Prevalence and resistance of the commensal flora in non-hospitalized patients

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Casper Dirk Johannes den Heijer

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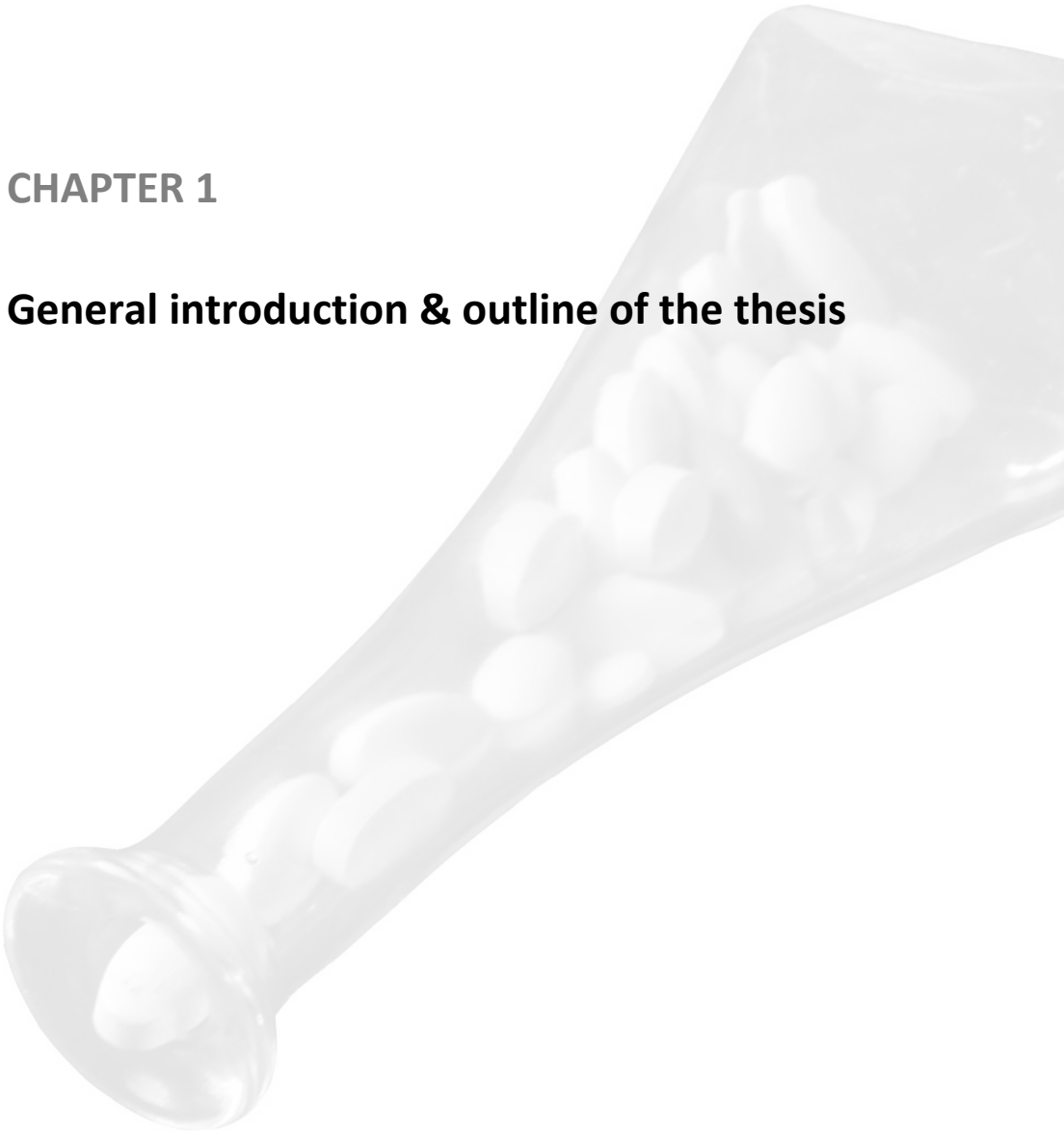

CHAPTER 1

General introduction & outline of the thesis

THE COMMENSAL FLORA

General

We now know that on almost every environmentally exposed surface of our bodies an abundance of microorganisms is to be found.[1] The combined membership of these microbial communities outnumbers our own cells by at least a factor 10.[2] Each community contains microorganisms from certain families and genera that are found in the same habitat in many or most individuals, although at the species and strain levels the microbiota of an individual can be as unique as a fingerprint.[3] Previously, it was believed that most bacteria could be seen as commensal, i.e. they would benefit from the host, while the host was unaffected. It is now becoming more and more clear, however, that the relationship between host and bacteria should be seen as mutualistic, i.e. both the bacteria and the host benefit.[4] These polymicrobial communities contribute profoundly to the structure and function of the tissues they inhabit and thus play an important role in the balance between health and disease.[1] Together, our ~100 trillion microbial symbionts (the human microbiota) provide us with crucial expertise; for example, we rely on them to assist us with nutrition, resistance to pathogens, and the education of our immune system.[2]

The current understanding of the human microbiota relies heavily on cultivation-based approaches and is therefore biased and incomplete.[3] With the initiation of the Human Microbiome Project (HMP) in 2007, which aims to characterize the microbial communities typical of human body sites, the number of known species has increased exponentially.[5] Although imperfect, molecular approaches that identify microorganisms from small-subunit (16S) ribosomal RNA gene sequences (as used in HMP) offer advantages over cultivation. The five major body sites targeted by the HMP are the skin, the gastro-intestinal (GI) tract, the oral cavity, the urogenital/vaginal tract and the respiratory tract.[5] For the scope of this thesis, I will focus on two of these sites, namely the skin and the GI tract.

Skin and nasal mucosa

The role of microbes on the skin surface has not been studied extensively, especially when compared with the intestinal flora.[4] Although clinical studies have produced valuable information about the number and types of microbes on the skin, their function has not been assessed. Presumed beneficial roles of resident microbiota include inhibition of pathogenic species and further processing of skin proteins, free fatty acids, and sebum.[6]

One of the best studied bacteria that can be isolated from the skin is *Staphylococcus aureus*, which is characterized by circular, golden-yellow colonies, β-haemolysis of blood agar and coagulase-positivity.[4] Most of the information on this bacterium, however, has been derived from studies related to the presence of *S. aureus* in the anterior nares of the nose, because this is the most frequent carriage site in humans.[7]

In general, nasal carriage of *S. aureus* is found in about one third of the subjects studied.[8] However, a considerable variability has been reported in this prevalence, ranging from 10% in an outpatient Turkish population to values above 50% in patients with insulin-dependent diabetes mellitus.[8,9]

This range in prevalence could be explained by the complexity of factors that determine *S. aureus* nasal carriage. Among these are general host characteristics, such as age, gender, ethnicity and the existence of co-morbidities (e.g. diabetes, HIV), but also genetic and immune host factors have been identified as contributing to the carriage status.[7,9] Environmental factors also appear to play a role: examples are hospitalization, type of occupation (e.g. healthcare workers) and living in large households (≥5 members).[7,10] Finally, bacterial factors have been thought to be implicated in the adherence of strains to the nasal mucosal flora and their protection from the immune response of the host, thereby promoting (sustained) carriage.[9]

The reason that so much effort is being put into the determination of *S. aureus* nasal carriage, is the report in numerous studies that carriers have a higher risk of becoming infected by *S. aureus* than non-carriers and, when infected, have a worse clinical outcome.[11] For example, in a Dutch study it appeared that nosocomial *S. aureus* bacteraemia was three times more frequent in *S. aureus* nasal carriers than in non-carriers, and *S. aureus* bacteraemia-related death was also more common in carriers.[12] In vulnerable hospitalized patients, such as HIV-infected patients or patients undergoing haemodialysis, *S. aureus* nasal carriage has also been identified as a risk factor for the development of nosocomial infections. In the community, most studies focused on the association between *S. aureus* nasal carriage and skin and soft tissue infections and the majority found a positive correlation.[7]

Gastro-intestinal tract

The gastro-intestinal tract, and especially the colon, is the body site with the largest number of microbes, namely ~10^{14} microbial cells.[13] For this reason, the microbiota of the intestines has received more attention from the research community than other body sites. This has resulted in the discovery of several functions of importance for the host. The microbiota can efficiently limit infection of the intestines by pathogenic bacteria.[14] In fact, during an infection, the microbiota may have at least three important functions: 1) it may block the growth of the pathogen and thus interfere with the infection right from the beginning ('colonization resistance'); 2) it may prime the host's innate and adaptive immune systems, thus preventing disease which would otherwise

be caused by the pathogen's virulence factors; 3) it can help in the clearance of the pathogen from the intestinal lumen at the end of an infection.[14]

The dominant bacteria found in the adult human large intestine belong to 2 major phyla, the Gram-negative Bacteroidetes and the Gram-positive Firmicutes, which together generally account for 90% of the total bacteria-based 16S rRNA-targeted molecular analyses.[15] Coliforms (*Escherichia coli*, Enterobacter species and other Gram-negative organisms) are the predominant facultative anaerobes, of which the Gram-negative rod-shaped *E. coli* is the most prevalent and has the greatest clinical significance.[16] The niche of commensal *E. coli* is the mucous layer of the colon.[17] There are, however, several highly adapted *E. coli* clones which have acquired specific virulence attributes that enable them to cause disease within or outside the gastrointestinal tract. Strains that can induce enteric or diarrhoeagenic disease are referred to as intestinal pathogenic *E. coli*, whereas strains leading to infections outside the intestines are named extra-intestinal pathogenic *E. coli* (ExPEC). The extra-intestinal site most frequently infected by *E. coli* is the urinary tract, resulting in the onset of urinary tract infections (UTIs).[18] UTIs are the result of the entry of uropathogens into the urinary tract and the most common route taken is via the urethra by bacteria that originate in the rectal flora, e.g. *E. coli* (the ascending route).[19,20]

The fact that, in approximately 80% of the uncomplicated UTIs in women, *E. coli* is isolated as the causative pathogen, and the estimation that 50% of all women will develop a UTI during their lifetime, show the impact of this bacterium.[21,22] In the Dutch general practice setting, it was estimated that 772,000 women had experienced a UTI in 2010.[23]

ANTIBIOTIC RESISTANCE

General

Since the discovery of penicillin, as first antibiotic agent against bacterial infections, antibiotic resistance has developed in many bacterial species.[24] Antibiotic resistance is defined as the non-susceptibility of a bacterium to a clinically relevant concentration of an antibiotic and/or the possession of a mechanism or property by a bacterium which will render the antibiotic ineffective.[25] Several mechanisms of antibiotic resistance can be distinguished, as will be discussed below.

Resistance mechanisms and means of transmission

Bacteria can be intrinsically resistant to an antibiotic, which means that a whole species or genus of bacteria is not susceptible to a particular drug, for example, the Gram-negative bacteria to vancomycin.[25]

Another means by which bacteria circumvent antibiotics is by acquiring resistance through spontaneous mutations or horizontal gene transfer (also known as lateral gene transfer or transposition) by processes of conjugation, transformation or transduction.[26] Conjugation is the only process in which genetic material is transferred between bacterial cells by cell-to-cell contact or through a bridge-like connection.[16] When exogenous DNA is taken up from the bacterium's surroundings through its cell membrane, the genetic alteration that is the result from the direct uptake, incorporation and expression of the new genetic material is called transformation.[16] Transduction is the process by which DNA is transferred from one bacterium to another by a virus, which is called a bacteriophage.[16] These processes of horizontal gene transfer provide the single most important mechanism that accelerates the spread of antibiotic resistance genes.[27] This is due to the location of the major part of the genes that mediate resistance on transferable plasmids or on transposons that can be disseminated by horizontal gene transfer. Plasmids are DNA molecules that are separate from a bacterium's chromosomal DNA and replicate independently.[16] Transposons are mobile pieces of DNA that can insert themselves into various locations on the bacterial chromosome, as well as into plasmids and bacteriophage DNA. Some transposons and plasmids have genetic elements termed integrons that enable them to capture exogenous genes. A number of antibiotic resistance genes may thus be inserted into a given integron, resulting in resistance to multiple antimicrobial drugs.[28]

Determinants

The previous paragraph has shown that bacteria have a wide range of tools at their disposal which enable them to acquire and disseminate antimicrobial resistance. Several factors have been identified which enhance these processes. These will be highlighted in the section below, with special attention being given to antibiotic use.

Antibiotic use

One of the most important drivers of antibiotic resistance is the use of antibiotics.[29,30] Hence, the frequency of acquired resistance is positively associated with the consumption of antimicrobial drugs.[26] This trend of higher antibiotic consumption = higher antibiotic resistance has been demonstrated for several antimicrobial agents.[30]

The relationship between antibiotic administration and resistance has resulted in a vicious circle in which the use of antibiotics leads to resistance, resistance results in treatment failure, and the medical community reacts by administering more antibiotics.[29] High use of antimicrobial agents will per se result in an increasing prevalence of antimicrobial resistance, but the incorrect use of these drugs has accelerated the rate of acquisition of resistance. Use of antibiotics in insufficient dosage kills only the susceptible organisms in the bacterial population, sparing the more resistant ones. Use of

the antibiotic at the correct dosage will kill the resistant bacteria also. In particular, inconsistent dosing or incomplete administration of a prescribed drug are problems that regularly arise.[18]

In this perspective also, the impact of non-prescribed antibiotics should be noted. In a recent review on this topic, it appeared that all over the world antibiotics were obtainable without prescription and accounted for 19–100% of all antibiotic use outside northern Europe and North America. It was noted that frequent non-prescription use is associated with increased antimicrobial resistance, probably because of the non-adherence to optimal dosing and treatment regimens.[31]

After (incorrect) antimicrobial treatment, which kills the susceptible bacteria, resistant organisms will fill the vacuum at both the individual level, including the site of infection and the person's commensal flora, and the community level.[29] As reported by Costelloe *et al.* in a well-conducted meta-analysis on the effect of antibiotic use on antibiotic resistance, this replacement was most marked in the month directly after prescription, but was detectable for up to 12 months. The authors stated that this residual effect is likely to be an important cause of the high endemic levels of antibiotic resistance in the community.[32] In line with this finding is the observation by Austin *et al.* that the time scale for emergence of resistance under constant selective pressure is much shorter than the decay time after cessation or decline in the level of drug use.[33] It also appears that consumption of antimicrobial agents not only disturbs the ecology of the flora of the treated patient, but of the whole household.[34]

Geographically, antibiotic use is significantly correlated with per capita gross domestic product (GDP), which is considered to be an indicator of a country's standard of living. This is probably due to poor access to antibiotics, or only access through informal channels, on account of poverty and poor infrastructure in countries with a relatively low GDP per capita. However, the United Nations reported recently that per-capita GDP is growing rapidly in many low per-capita GDP countries such as China and India, but also Brazil, Mexico and Turkey.[35] With the high population densities of these countries, antibiotic use is likely to increase worldwide in the coming years, with associated increased pressure on bacteria.

Within Europe, a clear trend can be observed of higher antibiotic prescription rates and resistance in the south as compared with the northern countries.[30] Recent data from the European Surveillance on Antimicrobial Consumption (ESAC) network in 20 European countries showed that outpatient antibiotic use in the Netherlands was 11.4 defined daily doses per 1000 inhabitants (DID – a standardized measure for antibiotic consumption). This was still relatively low compared with other European countries, although the Netherlands was one of 8 countries that showed an increase of more than one DID compared with 1997/1998 data.[36]

Differences in antibiotic consumption are not only observed between countries, variation in antibiotic prescribing rates has also been noted between general practices, which could not be explained by differences in patient population.[37]

Other determinants of antibiotic resistance

In addition to the selective pressure of antibiotic use, other determinants need to be considered in relation to the antibiotic resistance problem. Ingestion of resistant bacteria from food (meat, fruit and vegetables), due to the agricultural usage of antibiotics, is increasingly being recognized as a potential source of antimicrobial resistance.[24,38] Furthermore, resistant bacteria in animals can also be transmitted by direct contact.[39] Several studies have reported the isolation of antibiotic resistant bacteria from environmental samples, such as aquaculture, waste water and sludge. Some of these bacteria are genetically close to human pathogens, thus potentially contributing to the antimicrobial resistance problem in mankind.[40] Moreover, the increased opportunities to travel over long distances, have given bacteria a better chance of disseminating themselves over the world, as shown in several examples already reported in the literature.[41] Also contributing is the structure of society, including more and more locations with a high population density, such as day-care centres for children and nursing homes for the elderly, which enhance the chances of dissemination of antimicrobial resistance.[24,38] Other factors to consider are the increasing civil unrest, food shortages and natural disasters that leave vulnerable individuals in crowded conditions.[35]

Community versus hospital: change in perspective

For a long time, the hospital in general, and the intensive care unit in particular, were considered the most important reservoir of antibiotic resistant bacteria, because of the intensive antibiotic use in these environments.[29,38] Most surveillance studies addressing antimicrobial resistance were performed in the nosocomial setting, resulting in a largely unknown prevalence of resistance in the community.

With the emergence of resistance in common bacterial pathogens in the community, such as *Streptococcus pneumoniae* and *S. aureus*, came increased awareness of the outpatient setting being important for the development and spread of antimicrobial resistance. Indeed, there has been a change from hospital to community in the last two decades, with respect to the antimicrobial resistance problem.[38] Considering 80-90% of all antibiotics are being consumed within the community, this factor will at least partly be responsible for this shift.[30,38]

Commensal flora as reservoir of antimicrobial resistance

As mentioned earlier, resistance to antibiotics is the result of direct selection at the site of infection or in the commensal flora. The role of the commensal flora as a natural reservoir of bacterial resistance may even be more important than the infectious foci as the microbiota may repeatedly be affected by any antibiotic treatment, whether or not the drugs are used to treat a bacterial infection, whereas resistant pathogens

can emerge from infectious foci only in patients actually infected. In addition, larger numbers of bacteria and species are present, allowing multiple horizontal gene transfers to pathogens as well as direct infection by commensal bacteria.[42] This large pool of commensal bacteria also provides a greater chance of conferring complex resistance mechanisms, which have less chance to arise in subdominant species.[43] Moreover, an increase in antibiotic resistance is often first apparent in the commensal flora before affecting pathogenic bacteria.[44] The resistance pattern in the commensal flora is, therefore, a good indicator for future resistance problems to be expected in pathogens.

The role of commensal bacteria as a reservoir of antimicrobial resistance, together with their infectious potential, as shown for *S. aureus* and *E. coli* in previous paragraphs, make it important to monitor their presence in the population regularly, together with their antibiotic susceptibility patterns. By taking into account the resistance in the commensal flora, the problem of antibiotic resistance can be controlled at the source.[44]

Clinically important resistant bacteria

The capacity of *S. aureus* and *E. coli* to acquire resistance will be exemplified by multi-resistant forms of these bacteria, namely the meticillin-resistant *S. aureus* (MRSA) and the extended-spectrum beta-lactamase (ESBL)-producing *E. coli*.

Meticillin-resistant S. aureus

The potential of *Staphylococcus aureus* to acquire antimicrobial resistance was already demonstrated in the 1940s with the isolation of penicillin-resistant strains within two years of the introduction of this antibiotic. Twenty years later, a newly resistant strain emerged, the meticillin-resistant *S. aureus* (MRSA). MRSA isolates are resistant to all available penicillins and other beta-lactam antimicrobial drugs, thereby limiting the potential treatment options for induced infections substantially. The multi-resistance in MRSA is caused by the presence of the mecA gene, situated on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec).[27,45]

The evolution of MRSA is a good example of the shift from hospital to community, with initial isolation restricted to patients in the hospital setting and the subsequent emergence of MRSA infections in patients without exposure to health care systems.[26] MRSA strains obtained from the latter patients are often regarded as community-associated MRSA (CA-MRSA). Strains with their origin in the health care environment are named hospital-associated MRSA (HA-MRSA).

In general, HA-MRSA strains display a wider range of antimicrobial resistance than their community-associated counterparts, but seldom carry the genes for Panton-Valentine leukocidin (PVL), which is frequently found in CA-MRSA. In addition to the

genotypic differences, the populations affected also differ between the two forms of MRSA. CA-MRSA is mainly seen in previously healthy younger patients and causes predominately skin and soft-tissue infections (SSTIs), but has also been linked to several severe clinical syndromes such as necrotizing pneumonia and severe sepsis. HA-MRSA strains, on the other hand, have mainly been isolated from older patients (in health care settings) who have one or more co-morbidities. HA-MRSA strains tend to cause pneumonia, bacteraemia, and invasive infections.

With respect to the prevalence of MRSA, major differences are seen around the world, ranging from endemic levels in US hospitals to levels below 1% of *S. aureus* isolates from invasive infections in the Scandinavian countries and 1–5% in the Netherlands.[46-48] Even within Europe, there is a clear difference in prevalence, from the low percentages in Northern Europe mentioned above to a prevalence between 25-50% (Greece, Spain and Italy), or even ≥50% (Portugal), of invasive *S. aureus* isolates in Southern Europe.[47]

In high MRSA prevalence countries, such as England and the US, a promising trend has been observed in recent years, with less MRSA infections reported in England and some US hospitals. Improvements in infection control, mupirocin-based decolonization of patients and better management of intravenous lines, which may be a portal of entry, are factors contributing to this trend. In addition, several anti-MRSA drugs, such as linezolid and daptomycin, are being or have been developed.[49] These developments give hope for the future in the fight against MRSA, although these are still minor successes and continuous effort is needed to control the spread of this multi-resistant organism.

Extended-spectrum beta-lactamase (ESBL)-producing E. coli

With the focus of the pharmaceutical companies on the (successful) development of antimicrobial agents against Gram-positive bacteria over the past decade, there is currently a deficit in the treatment arsenal for multi-drug resistant Gram-negative bacteria. For this reason, the prospect is worse for infections caused by Gram-negative species, such as *E. coli*, than for those caused by Gram-positive bacteria. This concern with respect to the Gram-negatives is catalysed by a current rapid increase in their acquisition of a group of antimicrobial-resistant enzymes, known as the extended-spectrum beta-lactamases (ESBLs). ESBLs are defined as plasmid-encoded enzymes that can hydrolyze penicillins, oxyimino-cephalosporins of the first, second and third generation and aztreonam.[50] The success of their rapid spread can, at least partly, be explained by the fact that ESBL genes are generally found on plasmids, which can easily be transferred within and between species. On these plasmids other resistance genes are also often located (aminoglycosides, co-trimoxazole, quinolones), thus leading to co-resistance to multiple antibiotics within one bacterium.[51]

The ESBL-producing bacteria are responsible for a wide range of clinical presentations, from community-acquired urinary tract infections to severe nosocomial infections, such as ventilator-associated pneumonia.[51]

In the case of ESBLs also, most attention has been given to the increase in their presence in the hospital setting, but several reports have also indicated a community reservoir for these resistance genes. In this perspective, nursing homes and long-term care facilities are especially important, as reports of community-acquired ESBLs have been coming from these locations for several years.[51] What is also alarming, is that ESBLs have been isolated from healthy volunteers who had not been hospitalized or exposed to antibiotics within the previous three months.[52]

In Europe, resistance in invasive *E. coli* strains to third-generation cephalosporins, a proxy measure of ESBL presence, showed the lowest prevalence in the Scandinavian countries (1–5%) and the highest in Southern Europe (10–25%) in 2010 (Figure 1).[47]

For the Netherlands the prevalence ranged between these two extremes (5–10%). This prevalence seems to be increasing in the Netherlands, as was reported in a study performed in the Radboud University Nijmegen Medical Centre in 2008, with an estimated prevalence of 2.1% at that time among unselected clinical non-duplicate *E. coli* isolates.[53]

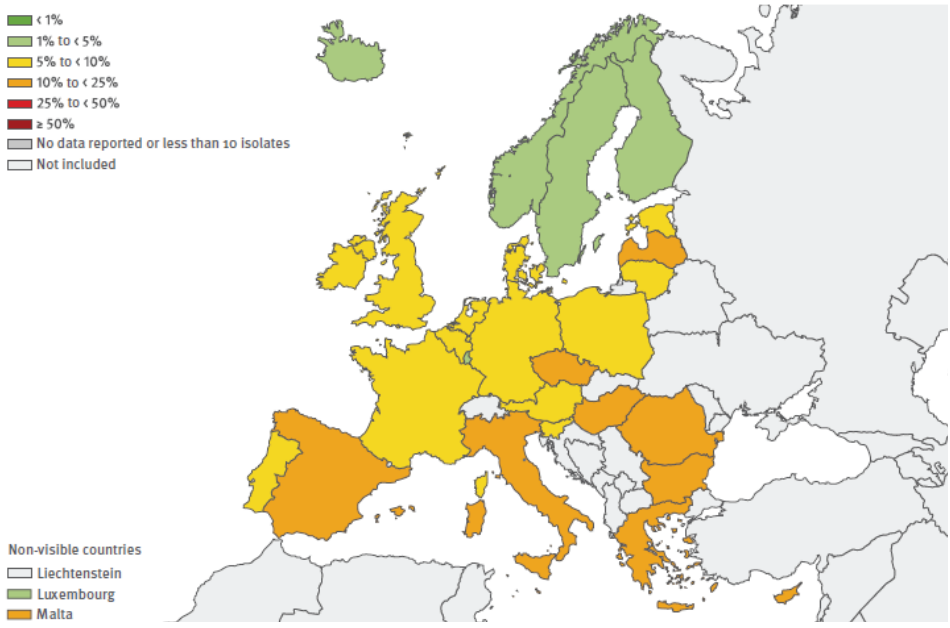

Figure 1. Proportion of invasive *Escherichia coli* isolates resistant to third-generation cephalosporines in 2010 (derived from EARS-net annual surveillance report 2010)[47]

Even more worrying is the emergence of resistance to carbapenems, which are drugs usually reserved for the most serious infections by multi-drug (Gram-negative) bacteria, as these are best able to circumvent at least some of the beta-lactam resistance mechanisms that affect most beta-lactam antibiotics.[51]

Impact of antibiotic resistance

Antibiotic resistance has a major impact on public health, because the development of antimicrobial resistance in *Staphylococcus aureus*, enterococci and Gram-negative bacilli is associated with increases in mortality, morbidity, length of hospitalization, and cost of health care.[54] For MRSA bacteraemia, a meta-analysis was performed including mortality data from 31 cohort studies conducted between 1980 and 2000, and this revealed a significant increase in mortality associated with MRSA bacteraemia compared with meticillin-susceptible *S. aureus* bacteremia (OR=1.93; P<0.001).[54] In a retrospective 1-year analysis performed in Italy, ESBL blood stream infections (BSI) led to a longer and more costly post-BSI-onset hospital stay and increased 21-day mortality as compared with non-ESBL BSI.[55]

The assumed reason for these negative consequences of antimicrobial resistance is the initial inadequate antibiotic therapy, leading to delayed optimal therapy, which leads again to a higher risk for the patient of acquiring life-threatening bacterial infections.[49]

The problem of emerging resistance has been traditionally solved by the search for, discovery of, and development of new antimicrobial classes, as well as by the improvement of already existing drugs. Unfortunately, it is becoming more and more clear that the development of antimicrobial agents cannot keep pace with the ability of bacterial pathogens to develop resistance.[29]

CONTROL OF ANTIBIOTIC RESISTANCE

General

In order to keep antibiotics effective in the future it is important to control antimicrobial resistance. There are several approaches to this goal, which can best be performed at the same time to get the optimal effect.[56] First, surveillance studies on antimicrobial resistance need to be performed on a regular basis, in order to obtain current data which can be translated to antibiotic treatment recommendations in clinical guidelines. Secondly, unnecessary antibiotic prescriptions should be reduced, e.g. prescribing an antibiotic to a patient with a viral or no infection. Thirdly, studies need to be conducted to determine the optimal regime and duration of an antibiotic for a specific disease. Fourth, the value of non-antibiotic alternatives, such as cranberries, lactoba-

cilli and tea tree oil needs to be investigated in order to objectify whether these are non-inferior treatment options for bacterial infections.[57-59] Finally, vaccination programmes can contribute to the control of antibiotic resistance. When the vaccine has a bacterial target, the advantage is obvious, but antiviral vaccinations can also be helpful, by reducing the potential complications of viral diseases, such as bacterial superinfections. In addition, the load of patients with symptoms of an infectious disease will be lowered, thereby decreasing the number of contacts between patients, which may lead to the prescription of an unnecessary antibiotic. In this thesis, I have focused mainly on the first two approaches, surveillance studies and improving clinical management, in order to reduce unnecessary antibiotic use. I elaborate on these two approaches in the following paragraphs.

Surveillance

Several important goals are the aim of antimicrobial resistance surveillance systems. Among these are the identification, interpretation and prediction of trends in resistance, the detection of new resistance mechanisms and the identification of outbreaks of resistant organisms.[60]

The necessity of local, national and international surveillance systems can be readily understood in the light of the above mentioned (high) potential of bacteria to transfer resistance and the variability in the resistance.

Essential for surveillance is the collection of reliable and consistent data, for which reference work, quality assurance and surveillance itself are important.[35] Reference work, performed by national or regional reference laboratories, involves the performance of a repertoire of confirmatory tests, including the molecular identification of genetic resistance determinants, to detect emerging resistance threats and rapid performance of confirmation tests for new resistance mechanisms. In addition, all participating laboratories within a surveillance network should regularly have an external quality assessment of their data, to evaluate their ability to identify antibiotic resistance of clinical and public health importance. Finally, a selection of organisms to be included in the surveillance should be made, which may differ according to ecological, socio-economical and epidemiological circumstances.

With the presence of reliable and consistent data, surveillance should serve as an early-warning system, the success of which depends on the rapid dissemination of its data to those involved.[60] In this way, a timely update of clinical guidelines can be achieved, which will increase the appropriateness of antimicrobial prescribing. For example, for urinary tract infections (UTIs) it has been suggested that antimicrobial agents should not be used for empirical treatment when resistance to this agent exceeds 20%.[61]

With respect to antibiotic resistance surveillance systems, several issues need to be considered. First, it is important to acquire surveillance data from different settings,

because antibiotic susceptibilities can vary considerably depending upon the patient population, e.g. gender, in the community, hospital, nursing home, etc.[62] This makes it inappropriate to extrapolate data from one setting to another.

Secondly, for bacterial infections, such as urinary tract infections, that can be treated empirically, i.e. without knowledge of the presence and the susceptibility pattern of a potential causative bacterium, most antibiotic resistant surveillance systems have the limitation that they only collect a selected number of clinical isolates (sampling bias). This selection is being made by the clinicians who often only send a patient's sample to the microbiological laboratory after initial treatment failure. The previous antibiotic pressure exerted on these patients leads to an overrepresentation of resistant organisms in these specimens. The use of unselected isolates, in which samples from all patients suspected of a particular condition are sent to the microbiological laboratory, is needed to reveal the true status of resistance in the patient population concerned.

Finally, it is essential to take into account the guidelines for antibiotic susceptibility, such as EUCAST and CLSI, being used when monitoring antibiotic resistance data over time.[63,64] Differences in cut-off values for antimicrobial resistance per species and per antibiotic between these guidelines and within these guidelines over time can bias the results, leading to inaccurate conclusions.

In addition to the fundamental importance of antibiotic resistance surveillance data, they can also be linked to the antibiotic usage in the patient population concerned or to supportive research programmes on infection control. In this way, the effect of antibiotic use on antibiotic resistance can be assessed and implemented infection control measures can be evaluated.

Optimal management

In clinical practice, optimal management of bacterial infections is an important factor in the containment of antibiotic resistance. In this respect, tools that reduce the diagnostic uncertainty of infectious diseases are vital. An accurate diagnosis of infections prevalent in a given setting can be especially helpful in reducing the burden of antibiotic resistance.

As noted earlier, a UTI is one of the most common bacterial diseases encountered in the general practice setting. With the empirical antibiotic treatment for UTIs mentioned above, great antibiotic pressure is exerted on the bacteria that can cause a UTI, the uropathogens. Hence, optimal management of this condition is highly relevant when considering the antimicrobial resistance problem.

In the evaluation of a patient suspected of a UTI, several factors need to be considered. First, there are general patient characteristics, such as gender, age, UTI history and co-morbidity, that relate to the distribution of uropathogens that may be found. In adult women without co-morbidity, 80% of the cystitis episodes are caused by *Es-*

cherichia coli, whereas this fraction is much smaller in UTIs in men, children and women with recurrent UTIs or complicating host factors, such as nephrological co-morbidities or diabetes mellitus.[65] These differing distributions have implications for the optimal empirical antibiotic management of these infections.

The clinical symptoms of the patient also need to be taken into account, with the presence of dysuria, urinary frequency and urgency as the classical symptoms of cystitis. The presence of fever (temperature above 38^{o}C), flank pain and malaise could indicate a UTI with tissue invasion, such as pyelonephritis or prostatitis.[66] GPs often use dipstick tests as a diagnostic instrument for UTIs. These tests indicate the presence of various substances in a patient's urinary sample and tests for nitrite and leucocyte-esterase are most often applied.

The evaluation of the predictive value of diagnostic information, i.e. patient characteristics, symptoms and dipstick results, can provide clinicians with a clinical decision rule aimed at improving the quality of care. For UTIs, McIsaac *et al.* have shown that the application of a simple diagnostic algorithm can result in the correct treatment of most women with UTI and can reduce unnecessary urine culture testing and antibiotic prescriptions.[67] In this perspective, it is not only necessary to detect the signs or symptoms to rule in a UTI, but also the factors that can help to exclude this diagnosis. Because the predictive power of the diagnostic information depends on the setting and the patient population involved, clinical prediction rules need to be developed per setting and per patient category. It is also important to validate these rules, because the performance on the initial patient sample often yields an over-optimistic result.[68]

The strength of this evidence-based antibiotic prescribing has also been shown for other conditions, such as lower respiratory tract infections, for which Cals *et al.* reported a reduction in antibiotic prescribing based on a point-of-care test for C-reactive protein.[69] Further measures that can be taken to improve antibiotic prescribing in general practice are enhancement of the communication skills of GPs or a multifaceted approach to promote prudent use of antibiotics by both health care workers (doctors) and consumers (patients) [37,56,69] These interventions can hopefully help to reduce the variation in antimicrobial prescriptions observed between general practices.

Finally, public health campaigns have been introduced to increase the awareness of the antibiotic resistance problem and to provide information on prudent antimicrobial use. Huttner *et al.* evaluated twenty-two of such campaigns and concluded that the majority of the campaigns that were formally evaluated appeared to reduce antibiotic use. The effect on antibiotic resistance could, unfortunately, not be assessed.[70]

OUTLINE OF THE THESIS

The aim of this thesis is to give insight into the prevalence of antibiotic resistance in the extramural setting for two (important) bacteria, *Staphylococcus aureus* and *Es-*

cherichia coli, and into the means of controlling their resistance to antibiotics. A literature review is given of the current knowledge on the commensal flora, antibiotic resistance, and in particular for *S. aureus* and *E. coli*, and means to control antibiotic resistance in **Chapter 1**. In **Chapter 2**, the set-up of the study 'The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) is described. In this study the Gram-positive bacteria *S. aureus* and *Streptococcus pneumoniae* were isolated from nose swabs taken from patients who contacted their GP with a non-infectious complaint. Nine European countries were involved in APRES, selected on the basis of geographical spread and differing levels of (extramural) antibiotic use. After the collection of 4,000 nose swabs per country, the prevalence of antimicrobial resistance was determined in the strains which had been identified and isolated. For *S. aureus*, the results are given in **Chapter 3**, including the MRSA prevalence found. In addition to the identification of MRSA, the population structure of these strains was also determined by means of *spa*-typing.

In the following chapters, the focus changes to one of the most prevalent bacterial infections in general practice, i.e. urinary tract infection (UTI). To control antibiotic resistance effectively, clinical guidelines need to be based on current resistance data and resistance trends should be monitored over time. For this reason, the antibiotic susceptibility was determined of *E. coli* strains which were isolated from urinary samples of patients who visited their GP in 2009 with symptoms suggestive of a UTI (e.g. dysuria, urinary frequency and/or urgency). Patients were recruited from general practices participating in NIVEL's Sentinel General Practice Network. These general practices have a patient population which is nationally representative for age, gender, regional distribution and population density. In this way, these data could be extrapolated to the whole Dutch population and so could be used in the update of the national UTI treatment guidelines. In addition, a similar study had been conducted in 2004, which made the monitoring of resistance over time possible, by comparing the 2004 and 2009 data (**Chapter 4**).

UTI in men are less well studied and often data from UTI studies performed among women are generalized to men. However, this generalization can be questioned because of the clear genito-urinary differences by gender (e.g. involvement of the prostate in men). Therefore, a similar study to that described in Chapter 4 was performed among men. *E. coli* resistance data between women and men were compared, and also resistance over time was investigated for male UTIs (**Chapter 5**).

In addition to up-to-date clinical treatment guidelines, the diagnostic approach to a bacterial infection is important in the control of antibiotic resistance. Optimization of the diagnostic process results in a greater chance of deciding correctly whether to treat a true bacterial infection or to withhold an antibiotic prescription when a bacterial infection is less likely. For this purpose, a clinical prediction rule for male UTIs was developed in order to optimize empirical antibiotic treatment (**Chapter 6**).

A patient population on which great antibiotic pressure is exerted is that of women with recurrent UTIs (rUTIs), as the standard treatment consists of low-dose antibiotic prophylaxis. This antibiotic regimen was compared with non-antibiotic alternatives (cranberries and lactobacilli) in the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study. The identification of a group of women with rUTIs who do not benefit from this prophylactic treatment, is another way of reducing the antibiotic load significantly. For this reason, the predictive value of asymptomatic bacteriuria (ASB) for the development of a (symptomatic) UTI within this patient population was determined, within the context of the NAPRUTI study. In addition, the question of whether an *E. coli* strain isolated from a ASB sample in the month preceding an *E. coli* UTI was predictive for the strain that caused the UTI, was studied by comparing antimicrobial susceptibilities and pulsed-field gel-electrophoresis (PFGE) patterns (**Chapter 7**).

Insight into the determinants of antimicrobial resistance could be helpful for the development of adequate actions to control it. In **Chapter 8**, these determinants have been investigated for *E. coli* in women with rUTI, again within the context of the NAPRUTI study. This was not only done for *E. coli* strains isolated from urinary samples, but also for strains from faecal samples, as faecal *E. coli* are considered the reservoir for strains causing a UTI. In this way, the determinants of *E. coli* resistance could also be compared between bacteria of urinary and faecal origin.

Finally, an overview of all the presented results are given and discussed in **Chapter 9**, together with recommendations for future research which may help in the control of antibiotic resistance.

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CHAPTER 2

The appropriateness of prescribing antibiotics in the community in Europe:
Study design

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ABSTRACT

Background

Over 90% of all antibiotics in Europe are prescribed in primary care. It is important that antibiotics are prescribed that are likely to be effective; however, information about antibiotic resistance in the community is incomplete. The aim of our study is to investigate the appropriateness of antibiotic prescribing in primary care in Europe by collecting and combining patterns of antibiotic resistance and antibiotic prescription in primary care. We will also evaluate the appropriateness of national antibiotic prescription guidelines in relation to resistance patterns.

Methods/Design

Antibiotic resistance will be studied in an opportunistic sample from the community in nine European countries. Resistance data will be collected by taking a nose swab of persons (n=4,000 per country) visiting a primary care practice for a non-infectious disease. *Staphylococcus aureus* and *Streptococcus pneumoniae* will be isolated and tested for resistance to a range of antibiotics in one central laboratory. Data on antibiotic prescriptions over the past 5 years will be extracted from the electronic medical records of general practitioners (GPs). The results of the study will include the prevalence and resistance data of the two species and 5 years of antibiotic prescription data in nine European countries.

The odds of receiving an effective antibiotic in each country will be calculated as a measure for the appropriateness of prescribing. Multilevel analysis will be used to assess the appropriateness of prescribing. Relevant treatment guidelines of the nine participating countries will be evaluated using a standardized instrument and related to the resistance patterns in that country.

Discussion

This study will provide valuable and unique data concerning resistance patterns and prescription behaviour in primary care in nine European countries. It will provide evidence-based recommendations for antibiotic treatment guidelines that take resistance patterns into account which will be useful for both clinicians and policy makers. By improving antibiotic use we can move towards controlling the resistance problem globally.

BACKGROUND

Resistance to antibiotics is a growing public health problem.[1-3] The prevalence of antibiotic-resistant micro-organisms in both hospitals and the community is increasing.[4-6] Several studies have demonstrated that resistance frequently leads to a delay in the administration of effective therapy, which may be associated with increased costs, morbidity or even mortality.[7,8]

A number of factors can explain the increasing trend in resistance but high exposure to antibiotics (which leads to a high selective pressure) is considered the most important cause.[9] Numerous individual and ecological studies have established a link between increased antibiotic consumption and the emergence of antibiotic resistance worldwide.[10-12] In Europe the most common exposure is the intake of antibiotic drugs, over 90% of which is prescribed in primary care. The variability of prescription rates is high: antibiotic use is low in northern, moderate in eastern and high in southern regions of Europe.[6]

While the pharmaceutical industry is running out of options to develop new antibiotics, a way to decrease the exerted selective pressure is to cautiously and appropriately handle antibiotic prescriptions.[13] Antibacterial drug use has to be both necessary and appropriate to minimize the development of antibiotic resistance. It is unnecessary when no antibacterial drug is indicated and inappropriate when antibacterial treatment is indicated but an incorrect agent is selected (inactive against the most likely causative pathogen).

To assess the appropriateness of prescribing antibiotics in primary care, knowledge about likely aetiological agents and their resistance patterns is required.[14] When General Practitioners (GPs) are provided with data about the types and prevalence of resistant pathogens in their own region or country, antibiotic prescription could be optimised.[15] However, most resistance research has been carried out in hospital settings, and well-documented information about community resistance patterns is limited.[4,16-19] To support GPs in optimal antibiotic prescribing, it is necessary to define and encourage appropriate antibacterial use by utilising national data and developing evidence-based guidelines.[20] This study aims to fill this gap in knowledge,[21] and will analyse the appropriateness of antibiotic prescribing in primary care. The main research question is: 'To what extent is the prescribing behaviour of primary care physicians in Europe congruent with the national or regional community antibiotic resistance patterns?'.

Our analysis is twofold: Firstly, we will determine community resistance patterns in nine European countries and link these to the prescription behaviour of GPs to assess their congruency. We hereby hypothesize that higher antibiotic prescription rates are associated with higher resistance rates. Secondly, we will evaluate the current

guidelines used by GPs with regard to the extent to which they are congruent with national resistance patterns, in order to make or revise recommendations, if necessary.

The resistance patterns and prescription patterns will be linked in this study to assess the appropriateness of prescribing antibiotics in Europe. We will specifically look at differences between countries, and as far as possible within countries. This article describes the design of this study and discusses the strengths and limitations of the study protocol. The results are expected at the end of 2013 and will be published in separate articles.

METHODS/DESIGN

Design overview

The research question will be answered using aggregated data in an ecological study design. We define the appropriateness of prescribing as the congruency between resistance patterns and prescription patterns; this will be operationalised as the odds that a patient will be given an effective drug. Data collection will be done within the APRES study (The appropriateness of prescribing antibiotics in primary care in Europe with respect to antibiotic resistance). The study will be conducted in nine countries in Europe: Austria, Belgium, Croatia, France, Hungary, the Netherlands, Spain, Sweden and the United Kingdom. We chose to include countries with different levels of prescription behaviour, to maximize our analysis range.

Resistance data

Study population

Practices

GP practices (20 per country) are recruited through an existing GP network in each country.[22] This network should be representative of the GPs in that country or region as much as possible, and a prerequisite is the ability to deliver electronic prescription data of the participating practices for the past five years. Geographical spread will be obtained by recruiting practices in both rural as well as urban areas. Some countries (Spain, Croatia) will also include primary care paediatricians as these are the ones that deliver primary care to children in these countries.

Patients

The study will be carried out in the primary care setting and the target population is the community-based without bacterial infections as a proxy for the general population. We use an opportunistic random sample of visitors of general practices. In the waiting room information leaflets and posters are used for recruitment purposes. All countries use the same inclusion criteria:

- No antibiotic use in the past 3 months
- No hospitalization in the past 3 months
- Registered patient or regular visitor of the practice for at least 1 year
- Age 4 years and older (UK: 18 and over)
- No residents of nursing homes
- No existing infectious disease at time of visit for which antibiotics are prescribed
- Not immunocompromised
- No terminal illness
- No out of hour consultations

During the period of data collection (November 2010 – May 2011) each practice recruits 200 patients to participate in the study. A Dutch study with the same design proved this to be feasible.[23] The data collection is spread across several months to maximize the potential detection of *S. pneumoniae*.[24]

Whether a patient meets the inclusion criteria will be assessed by the GP or qualified practice nurse. Most criteria are apparent at first sight; others are asked when the patient is invited to participate. Regarding age and gender, we strive for an equal stratification along the different groups; male, female and children (4-19), middle age (20-65) and elderly (>65 years old). Recruitment will be monitored with a two-weekly update about the recruitment.

Resistance patterns

A nasal swab is taken from all participants by the GP or qualified practice nurse. The swabs are sent to one laboratory in each country using special envelopes. They should arrive within 48 hours, to increase the survival rate of *S. pneumoniae*. As a consequence of the ethical approval procedure, in the UK the nasal swabs are taken by the patients themselves, at home. All patients from whom a nasal swab is taken fill in a short questionnaire regarding their background and confounding variables. The national laboratories will isolate the bacteria (*Staphylococcus aureus* and *Streptococcus pneumoniae*) from the swabs. The isolation and identification procedures are depicted in Table 1. These bacteria were selected because of their high impact on health care.[25]

Prior to the swabbing period, the national laboratories are evaluated regarding the quality of their isolation procedures.

Table 1. Laboratory protocol for the isolation and identification of *S. aureus* and *S. pneumoniae*

	Preparation of the swab	Incubation	Morphology	Identification tests	Storage of isolates
S. aureus	Plate nasal swab on standard blood agar plate **and** selective agar plate (blood agar + gentamicin)	For 18-24 hours at 35°C in a CO_2-enriched atmosphere	Small to middle sized (β-hemolytic) white-yellow colonies	Coagulase (clumping/tube) **or** Latex agglutination	In skimmed milk cryovials (in duplicate)
S. pneumoniae	Plate nasal swab on standard blood agar plate **and** selective agar plate (blood agar + gentamicin)	For 18-24 hours at 35°C in a CO_2-enriched atmosphere	Small α-hemolytic colonies; umbilicate, transparent, flattened or teardrop-shaped (characteristic central depression)	Optochin <u>In case of doubt:</u> Bile esculin/-solubility **or** API **or** VITEK	In skimmed milk cryovials (in duplicate)

At the end of the data collection period, the isolated bacteria will be sent to the Department of Medical Microbiology of Maastricht University, the central laboratory of the project where the resistance testing will be performed. The antibiotic susceptibility of the isolated strains will be determined quantitatively using a broth dilution method in micro-titre plates according to the EUCAST standard.[26] The compounds tested include penicillins with and without beta-lactamase inhibitors, cephalosporines, macrolides, tetracyclines, quinolones, trimethoprim-sulfamethoxazole and rifampicin. For *S. aureus* also aminoglycosides and topical agents (fusidic acid and mupirocin) will be tested. This analysis will result in a resistance rate for every bacterium – antibiotic combination.

Sample size calculation

In a recent study among 2,000 community-dwelling persons in the Netherlands, 23% showed to be carrier of *Staphylococcus aureus,* whereas 4% showed to be resistant against antibiotics.[23] Power analysis showed that using a one-sided t-test (alpha=0.05) testing for a 50 percent difference (4% versus 6%) between countries with a power of 0.95, 1,000 isolates are needed. For 1,000 isolates, 4,000 swabs have to be taken in

each country. Therefore, for nine countries the total data collection will consist of 36,000 swabs.

Prescription data

Study population

The GPs and GP practices who are participating in swabbing the patients also take part in the collection of prescription data.

Measurements

We will collect data about all prescribed antibiotics from the participating practices for the past 5 years (i.e. calendar years 2006 to 2011). For every prescription of antibiotics, i.e. antibacterials for systemic use according to the Anatomical Therapeutic Chemical (ATC) classification (ATC code J01), we will collect the following information:

- Date of the prescription
- Diagnosis for which the antibiotic has been prescribed (if available)
- Identification of the chemical substance (7 digit ATC code)
- Number of packages (if available)
- Number of Defined Daily Doses (DDD's, if available)

The diagnostic data will be converted to the International Classification of Diseases, 10th revision (ICD-10).[27] No additional data collection is needed; all these data are routinely recorded in the patients' electronic medical records.

Data on characteristics of the practice population (the epidemiological denominator) including the size of the practice population and its age (in 5-year age bands) and gender distribution will also be collected. In countries with a registered list system, this file can be retrieved from the practice computer. In countries without a registered list system, estimates of the size and composition of the population will be provided[28] either based on characteristics of the patients visiting the practice, or based on extrapolation of local or regional population characteristics.[29]

Finally, each practice will provide information on a few practice characteristics in a short questionnaire (geographical area, number of listed patients, number of physicians working in the practice, age of the physicians).

Guidelines

In addition to the aforementioned data collection, relevant antibiotic treatment guidelines used in the nine participating countries will be collected and evaluated in relation

to the resistance patterns in each country. In particular, we will focus on syndromes and diseases mainly caused by *S. aureus* and *S. pneumoniae*: skin infections and pneumonia respectively. In cooperation with the GP networks in the countries, we will assess the most frequently used primary care guidelines on both content (specific recommendations) and quality. For the latter we will use a standardised instrument, with a focus on the evidence base of the recommendations.[30-33]

By comparing the current resistance patterns with the primary care guidelines per country, we will be able to make recommendations to improve these guidelines, incorporating the evidence we will collect.

Ethical approval

In every participating country ethical approval for this study has been obtained. All participants sign an informed consent form. For children a separate form is developed, where consent from one of their parents or their guardian is obtained. Children are not allowed to participate in the UK due to ethical restrictions.

DATA ANALYSIS

Descriptive analyses will be carried out to calculate the resistance patterns and patterns of prescribed antibiotics in each country. For countries where diagnostic data are available, more in-depth analyses will be carried out to calculate the appropriateness of antibiotics prescribed for specific diseases. Age and gender specific analyses will be carried out if the sample size allows for meaningful analyses. In general, women, children and elderly have a higher health care utilisation than other groups, and therefore have a higher a priori risk of having antibiotics prescribed. Consequently, they may show higher rates of resistance and/or other resistance patterns. The frequency of prescribed antibiotics will be expressed as a rate per 1000 listed patients. The distribution of the different types of antibiotics will be calculated as proportions of all antibiotics prescribed (the 'market share').

To test our hypothesis and analyse the congruency of the resistance and prescription data, we will calculate the odds of receiving an effective antibiotic on a national level. This 'effectiveness rate' will be calculated per country by multiplying the "market share" in prescriptions of a specific antibiotic with the established antibiotic resistance rate of the bacterium (e.g. if 50% of the prescribed antibiotics is penicillin and 20% of all bacteria is resistant to penicillin, penicillin's effectiveness rate is 50% x (100%-20%)=40%). The effectiveness rate will be calculated per country for each combination of antibiotic and bacterium. The sum of the effectiveness rates is an indicator for the appropriateness of prescribing antibiotics in each country.

Since we use hierarchical data, we will use a multilevel design to assess this appropriateness. Data are analysed with STATA and MLwiN for multilevel modelling. Potential confounders will be included in the model to adjust for these variables.

DISCUSSION

Strengths and limitations

This study will provide data from nine European countries on community-based resistance patterns and antibiotic prescription patterns in primary care, thus assessing the appropriateness of prescribing antibiotics. Our integrated data set, with a range in prescription and resistance patterns, is powerful and fills the gap in knowledge concerning community resistance patterns. We will not only provide information on resistance patterns, but also relate them to prescription behaviour and treatment guidelines. To improve the quality of prescribing it is important to monitor antimicrobial resistance and usage data on a national level and benchmark these by comparisons with other countries.[34]

Our first intent was to measure resistance in a random sample of the general population. Due to practical reasons this proved not to be feasible. The opportunistic sampling method that was chosen instead is potentially prone to selection bias. With our stratification methods, we will be able to reduce this bias and be able to conduct analyses concerning several age groups. In the UK, we are using a different design, where patients swab themselves. This may lead to a specific selection of patients. However, since we also measure background variables we are able to compare the participants' characteristics with those in other countries. Also, since we only measure at one point in time we cannot indicate a causal relationship between prescription behaviour and resistance patterns. However, our study will produce valuable data on a public health level.

Implications

Our consistent design throughout Europe provides an important insight into the appropriateness of prescribing antibiotics. With our results, we will be able to make evidence-based recommendations for antibiotic treatment guidelines. Clinicians and policy makers can both profit from this knowledge. By improving antibiotic use and decreasing resistance at local levels, we can move towards controlling the resistance problem in Europe. It would be valuable to continue monitoring resistance patterns in the community in the future.

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CHAPTER 3

Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *Staphylococcus aureus*, in nine European countries:

A cross-sectional study

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ABSTRACT

Background

Information on the prevalence of *Staphylococcus aureus* resistance has mainly been obtained from invasive strains, although the commensal flora is considered an important reservoir of resistance. Within 'The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) study, we compared the prevalence of nasal *S. aureus* carriage and antibiotic resistance, including meticillin-resistant *S. aureus* (MRSA), among healthy patients between nine European countries. The genotypic structure of the isolated MRSA strains was determined by means of *spa*-typing.

Methods

Nasal swabs were obtained from patients recruited by general practitioners (GPs) participating in existing nationwide GP networks. Swabs were sent to national microbiological laboratories for identification and isolation of *S. aureus*. Antibiotic resistance testing was performed at one central microbiological laboratory.

Findings

S. aureus was isolated from 6,956 out of 32,206 patients swabbed (21.6%). The adjusted *S. aureus* prevalence ranged from 12.1% (Hungary) to 29.4% (Sweden). Except for penicillin, the highest resistance was observed to azithromycin: range 1.6% (Sweden) to 16.9% (France). In total, 91 MRSA strains were isolated, with the highest MRSA prevalence being found in Belgium (2.1%). 53 different *spa* types were detected, with t002 (n=9) and t008 (n=7) being most prevalent.

Interpretation

The prevalence of *S. aureus* nasal carriage differed between nine European countries, even after correcting for age, sex and GP. In general, the prevalence of resistance, including MRSA, was low. MRSA strains found showed genotypic heterogeneity, both within and between countries.

INTRODUCTION

Staphylococcus aureus forms part of the human microbiota and the most consistent body site from which it can be isolated is the anterior nares.[1,2] In addition to the commensal role of this bacterium, *S. aureus* is capable of causing various types of human infections, ranging from minor skin infections to severe pneumonia.[3]

The potential of *S. aureus* to acquire resistance has been exemplified by the quick emergence of resistance to penicillin and meticillin. Especially the high levels of meticillin-resistant *S. aureus* (MRSA) reported over the last decades have been a cause of concern due to the paucity of alternative treatment options. At present, MRSA is the most important cause of antibiotic-resistant healthcare-associated infections worldwide.[4] The main driver for antibiotic resistance is considered to be antibiotic use.[5]

Until now, studies on the prevalence of *S. aureus* resistance have mainly focussed on invasive isolates, often obtained from hospitalized patients with bloodstream infections.[6] Due to the paucity of resistance data from the outpatient setting, the prevalence of resistance in invasive strains has been translated into guidelines for empirical antibiotic treatment of community-acquired infections, which will promote excessive use of broad-spectrum antibiotics in outpatients.[6] The commensal flora of a person is considered to be the reservoir for antibiotic resistance genes and, therefore, resistance in this flora usually precedes the acquisition of resistance in pathogens.[7] For these reasons, the resistance levels of the commensal flora should be taken into account in clinical guidelines for general practitioners (GPs) on the control of antibiotic resistance at the source.[8]

Within 'The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) study, one of the main objectives was to establish the prevalence of antibiotic resistance to commensal *S. aureus* in nine European countries with different levels and patterns of antibiotic prescription behaviour.[9] In this article, we assessed whether differences existed in the prevalence of nasal *S. aureus* carriage found in the nine participating countries. We evaluated the prevalence of antibiotic resistance among the isolated *S. aureus*, including levels of MRSA, in order to get an indication of the selection pressure that is exerted on the general population by antibiotics.[8] In addition, the population structure of the isolated MRSA strains was determined by means of *spa*-typing to detect possible clustering of specific MRSA strains within one or more countries.

METHODS

The APRES study design has been described in a previous publication.[9] For this reason, we shall only briefly mention the methods used in this study.

General practices and patients

The participating GPs (n=20 per country was aimed at) were recruited from existing national GP networks in Austria, Belgium, Croatia, France, Hungary, Spain, Sweden, the Netherlands, and the United Kingdom (UK) from November 2010 to August 2011. Each GP was asked to collect nasal swabs from 200 patients, aged ≥4 years (UK: ≥18 years due to ethical committee constraints), who visited his/her practice for a non-infectious condition. Patients who had been prescribed antimicrobial agents or who had been hospitalized in the previous three months were excluded, as well as immunocompromised patients (e.g. diabetes mellitus) and nursing home residents. The exclusion criteria are intended to guarantee the participation of "healthy patients" as a proxy for the general population. In addition to the collection of a nasal swab, the participant's background information (i.e. age and sex) was noted.

By means of a fortnightly recruitment update, equal stratification was sought with respect to sex and age groups, i.e. children (4–19), adults (20–64) and elderly (≥65 years old).

Ethical approval for the study was obtained in all participating countries and all patients provided written informed consent prior to inclusion.

Swabs

Before the start of the study, participating GPs received a protocol on the sampling of the nasal swabs, which was further explained to them by the national GP coordinator or study nurse. Both anterior nares of the patients were swabbed using a charcoal swab (Copan Italia, Transystem, cod. 114 C). The collected swabs were sent to one (national) microbiological laboratory for isolation and identification of *S. aureus*. All laboratories participating in the APRES study used a standardized protocol for the identification of *S. aureus*.[9] Before the start of the study, the quality of the *S. aureus* identification procedures of each laboratory had been evaluated by means of sending a blind panel of swabs, from which the identification results were reported back to the microbiological laboratory of Maastricht University Medical Centre (MUMC), the Netherlands.

Antibiotic resistance testing

When the analysis of the collected swabs was completed by the national laboratory, the isolated *S. aureus* strains were frozen and sent batchwise to the microbiological laboratory of Maastricht University Medical Centre (MUMC), the Netherlands, for antibiotic resistance testing. The resistance of *S. aureus* strains was determined, using the microdilution method, for the following antibiotics: azithromycin, ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, linezolid, oxacillin, penicillin, tetracycline, co-trimoxazole, and vancomycin. *S. aureus* ATCC 29213 was used as control strain. Methods were in accordance with the EUCAST guidelines and EUCAST epidemiological cut-offs were used as resistance breakpoints.[10]

All isolates susceptible to clindamycin and resistant to erythromycin were tested for inducible clindamycin resistance by means of the D-test.[11] In the case of a positive D-test, strains were considered resistant to clindamycin.

Meticillin-resistant *Staphylococcus aureus*

All isolates with MIC-values ≥1 mg/L to oxacillin were analysed for the presence of the mecA gene using a real-time PCR.[12] A real-time PCR for the *S. aureus*-specific genes femA and SA442 served as internal control.[3]

Real-time amplification of the *spa* locus, followed by sequencing, was performed as described previously.[13] The *spa* types were clustered into *spa*-CCs using the algorithm based upon repeat pattern (BURP) with the Ridom StaphType version 1.5 software package (http://www.ridom.de). The default settings recommended by the manufacturer were used.

Statistical analysis

To control for the known influence of age and sex on the prevalence of *S. aureus* nasal carriage[1] and the possible clustering of *S. aureus* carriage at a GP level, we calculated the *S. aureus* prevalence for each country using a multilevel logistic regression model. The multilevel model had three levels (country, GP and patient) and estimated the country prevalence of *S. aureus* based on the age and sex sample structure (the total sample population). The multilevel analysis was performed using the software package MLwiN.[14]

Because UK participants had a minimum age of 18 compared with age ≥4 for the other participating countries, the adjusted *S. aureus* prevalence was calculated twice. First, with all participants of all ages (without UK participants) providing the age and sex sample structure, and secondly, with all study participants aged 18+ (including the UK).

In calculating the MRSA prevalence, both the number of isolated *S. aureus* strains and the total study population were used as denominator.[15]

Multidrug-resistance (MDR) was defined as a strain being resistant to three or more antibiotic classes.[16] For this reason, azithromycin, erythromycin and clindamycin were grouped together in the calculation of the number of antibiotic classes to which *S. aureus* was resistant.

PASW version 18.0 was used for the statistical analyses and $P<0.05$ was considered statistically significant.

RESULTS

Patients

The number of patients recruited ranged from 3,132 (Belgium) to 4,017 (Hungary) leading to a total of 32,770 patients. Patients who did not meet the age inclusion criterion (aged <18 years in the UK or <4 years in the other participating countries), incompleteness of data (missing patient background or lab data) or incorrect sampling (no bacterial growth observed at lab analysis) was noted in 564 cases (1.7%, 95% confidence interval (CI): 1.6–1.9); range: 0.3% (0.2–0.5) in France and Spain to 4.2% (3.7–4.9) in Hungary. In Table 1 the baseline characteristics of the APRES patient population are given. Thus, 32,206 patients were included for analysis.

Table 1. Baseline characteristics of the participating patients

	Total patient population (n)	Excluded from analysis (n)			Women (%)	Age distribution (%)		
		Age[a]	Mis-match[b]	Incorrect sampling[c]		4–19 years	20–64 years	65+
Austria	3,380	4	56	11	56.6	5.3	72.3	22.4
Belgium	3,132	6	101	0	54.2	5.2	57.9	37.0
Croatia	4,013	1	35	17	59.4	16.0	53.9	30.1
France	3,870	0	12	0	55.0	9.5	64.6	25.9
Hungary	4,017	3	17	150	55.0	28.2	50.4	21.3
The Netherlands	3,873	14	12	0	57.1	10.3	63.8	25.9
Spain	4,001	0	11	0	58.6	12.0	57.2	30.8
Sweden	3,273	33	26	0	57.4	12.6	54.9	32.5
UK	3,211	3	52	0	59.2	1.6	69.1	29.3
Total	32,770	64	322	178	57.0	11.6	60.3	28.2

[a] Number of patients who did not meet the age inclusion criterion, i.e. aged <18 years in the UK and <4 years in the other participating countries.
[b] Defined as the absence of patient background information or lab data.
[c] Defined as the absence of bacteria (including normal nasal flora) on the agar plate at lab analysis.

S. aureus prevalence

The overall crude prevalence of *S. aureus* nasal carriage was 21.6% (n=6,956). Increasing age was associated with a lower *S. aureus* prevalence (overall odds ratio (OR)=0.992, 0.991–0.993). In addition, men had a greater chance of nasal carriage of *S. aureus* (overall OR=1.38, 1.31–1.46) than women. The adjusted *S. aureus* prevalence was highest in Sweden (29.4%) and lowest in Hungary (12.1%) (Table 2).

Table 2. Unadjusted and adjusted *S. aureus* prevalence by country

	Swab tested (n)	Prevalence of *S. aureus* nasal carriage (% [95% CI])		
		Unadjusted	Adjusted	
			All ages[a]	Aged 18+ only[b]
Austria	3,309	16.6 (15.4–17.9)	16.2 (13.2–19.8)	15.7 (12.7–19.2)
Belgium	3,025	19.3 (17.9–20.8)	19.4 (15.9–23.4)	18.8 (15.3–22.9)
Croatia	3,960	20.0 (18.8–21.3)	19.4 (16.0–23.3)	18.5 (15.1–22.5)
France	3,858	22.7 (21.4–24.0)	21.9 (18.2–26.2)	21.1(17.4–25.4)
Hungary	3,847	14.1 (13.0–15.2)	12.7 (10.3–15.6)	12.1 (9.7–15.1)
The Netherlands	3,847	27.9 (26.5–29.3)	27.3 (22.9–32.1)	26.3 (22.0–31.3)
Spain	3,990	19.3 (18.2–20.6)	18.8 (15.6–22.6)	17.3 (14.2–21.0)
Sweden	3,214	29.8 (28.2–31.4)	29.4 (24.7–34.5)	29.4 (24.6–34.8)
UK	3,156	25.8 (24.3–27.3)	[c]	25.4 (21.0–30.3)

UK, United Kingdom.

[a] All participants of any age (excluding UK participants) were used as the standard population.

[b] All participants aged 18+ (including UK participants) were used as the standard population.

[c] Not applicable to the UK as no patients under the age of 18 were included due to ethical committee constraints.

Antibiotic resistance

Antibiotic resistance results were available for 6,908/6,956 (99.3%) of the *S. aureus* isolates. Resistance was most common to penicillin, with almost 3 out of 4 isolates resistant to this agent (73.2%, 5,056/6,908). Apart from penicillin, macrolide (azithromycin and erythromycin) and clindamycin resistance showed the greatest variability between the countries with the highest resistance, France (16.8%) and Belgium (14.6%), and the lowest resistance: Sweden for both groups (1.6% and 1.5%, respectively). For all other agents tested, antibiotic resistance was below 10% in all participating countries (Table 3).

When the MIC distribution of azithromycin was investigated, all countries showed a peak at MIC=1 mg/L and more than 78% of azithromycin-resistant *S. aureus* was highly resistant (MIC ≥32 mg/L) to this agent in all countries.

Table 3. Country with lowest and highest prevalence of antibiotic resistance of commensal *S. aureus* with their national outpatient antibiotic use[a]

	Lowest antimicrobial resistance			Highest antimicrobial resistance		
	Country	Prevalence (% [95% CI])	Antibiotic use (rank)[a]	Country	Prevalence (% [95% CI])	Antibiotic use (rank)[a]
Azithromycin	Sweden	1.6 (1.0–2.6)	0.63 (1)	France	16.9 (14.6–19.6)	4.15 (9)
Ciprofloxacin	Sweden	0.6 (0.3–1.4)	0.79 (2)	UK	5.2 (3.8–6.9)	0.48 (1)
Clindamycin	Sweden	1.5 (0.9–2.4)	0.63 (1)	Belgium	14.6 (12.0–17.7)	2.96 (5)
Erythromycin	Sweden	1.6 (1.0–2.6)	0.63 (1)	France	16.5 (14.2–19.1)	4.15 (9)
Gentamicin	Sweden, France	0 (0–0.4)	[b]	Austria	2.2 (1.3–3.8)	[b]
Penicillin	Austria	64.8 (60.8–68.7)	7.09 (4)	Spain	87.1 (84.6–89.3)	12.31 (7)
Tetracycline	Spain	1.8 (1.1–3.0)	0.60 (1)	Croatia	7.2 (5.5–9.2)	1.57 (4)
Co-trimoxazole	Sweden	0 (0–0.4)	0.54 (5)	UK	1.0 (0.5–1.9)	1.18 (9)

UK, United Kingdom.

[a] The national outpatient antibiotic use per antibiotic class according to the European Surveillance of Antimicrobial Consumption (ESAC) data, expressed in defined daily dosage per 1000 inhabitants and per day.[17] The national outpatient antibiotic use of all participating countries are ranked, in which rank 1 = country with the lowest antibiotic use and rank 9 = country with the highest antibiotic use.

[b] The national outpatient use of aminoglycosides is not given by ESAC.

No resistance was found to linezolid and vancomycin, 3 isolates were resistant to daptomycin (2 Dutch, 1 UK).

Table 4. Number of antibiotic classes to which *Staphylococcus aureus* was resistant by country

	n	0 classes	1 class	2 classes	≥3 classes[a]
Austria	549	29.9	55.7	12.4	2.0
Belgium	582	20.1	64.9	12.9	2.1
Croatia	755	20.0	67.8	9.4	2.8
France	874	19.1	67.4	11.6	1.9
Hungary	539	19.5	65.7	12.1	2.8
The Netherlands	1,073	28.5	61.5	8.0	2.0
Spain	769	10.8	75.6	11.6	2.1
Sweden	955	33.2	63.6	3.0	0.2
UK	812	23.0	63.4	10.6	3.0

UK, United Kingdom.

[a] A strain that was resistant to three or more antibiotic classes was considered multidrug-resistant.

Values are given in %.

Multidrug-resistance occurred most frequently in the UK (3.0%, n=24) and the least in Sweden (0.2%, n=2) (Table 4). Six isolates were resistant to five antibiotic classes (Bel-

gium, Croatia, Netherlands (n=2) and the UK (n=2)) and one *S. aureus* was resistant to six classes (Croatia).

MRSA

A total number of 91 MRSA isolates was found, ranging from zero in Sweden to 16 isolates in France. When the number of isolated *S. aureus* was used as denominator in calculating MRSA prevalence, Belgium showed the highest prevalence (2.1%). Based on the total study population per country, Belgium, Croatia, France and the UK had the highest MRSA prevalence (0.4%) (Table 5).

Table 5. Prevalence of MRSA and *spa* types found by country

	MRSA prevalence			*Spa* types found
	Total (n)	% of *S. aureus* strains (95% CI)	% of total study population (95% CI)	
Austria	8	1.5 (0.7–2.8)	0.2 (0.1–0.5)	t003, t005 (n=2), t008, t010, t015, t034, t127
Belgium	12	2.1 (1.2–3.6)	0.4 (0.2–0.7)	t008, t011 (n=2), t038, t062, t231, t447 (n=3), t1923, t2346, t10847
Croatia	15	2.0 (1.2–3.3)	0.4 (0.2–0.6)	t002, t003, t005, t011, t014, t015, t045, t050, t127, t330, t535, t550, t2018, t5933, t10807
France	16	1.8 (1.1–3.0)	0.4 (0.3–0.7)	t002 (n=3), t008 (n=5), t010, t121, t622, t681, t777 (n=2), t2054, t5708
Hungary	8	1.5 (0.8–2.9)	0.2 (0.1–0.4)	t002 (n=2), t032, t127 (n=2), t330 (n=2), t1218
The Netherlands	9	0.8 (0.4–1.6)	0.2 (0.1–0.4)	t011 (n=2), t034, t038, t108, t267, t740, t1457, t10812
Spain	10	1.3 (0.7–2.4)	0.3 (0.1–0.5)	t002 (n=2), t022, t230, t846, t1081, t1203, t1610, t10814
Sweden	0	0 (0–0.4)	0 (0–0.1)	None
UK	13	1.6 (0.9–2.7)	0.4 (0.2–0.7)	t002, t020, t025, t032, t127 (n=2), t223, t852, t1214, t2436, t5414, t7922

MRSA, meticillin-resistant *Staphylococcus aureus*; UK, United Kingdom.

There was no evidence of clustering of MRSA strains in a GP practice in any country; the only incidence was two Dutch MRSA strains with *spa* type t002 originated from patients from the same GP practice. All other similar *spa* types per country belonged to patients from different GP practices.

Fifty-three different *spa* types and two non-typeable strains were found among the 91 MRSA isolates (Table 5). *Spa* type t002 was the most commonly found among

the MRSA isolates (n=9), followed by t008 (n=7). 70/91 (77%) strains could be clustered into 8 *spa*-CCs, 16 were classified as singletons and three strains were excluded because the *spa* types found were less than 5 repeats in length (Figure 1). All MRSA strains belonging to *spa*-CC 011 (n=10) were resistant to tetracycline, as against 17.3% (14/81) of the non-*spa*-CC 011 MRSA (OR=1.71, 1.22–2.40).

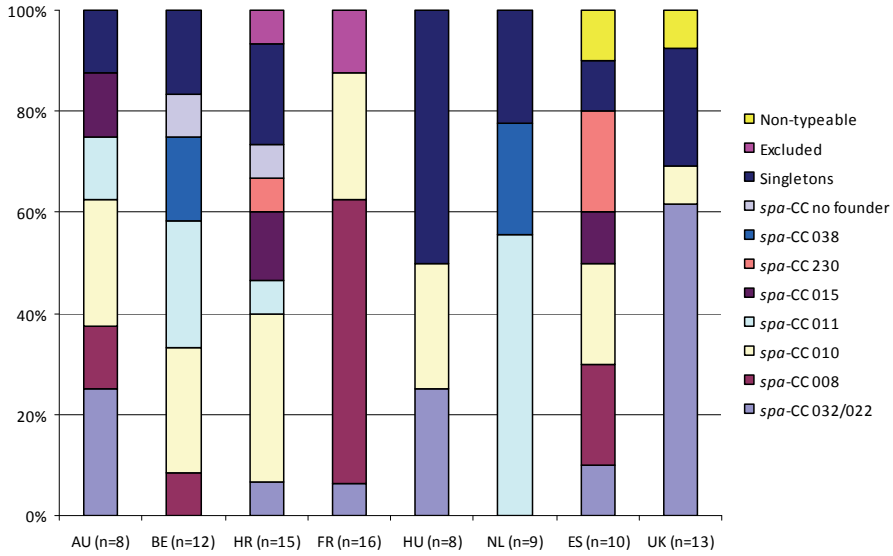

Figure 1. Distribution of *spa*-CCs by country

AU, Austria; BE, Belgium; HR, Croatia; FR, France; HU, Hungary; NL, the Netherlands; ES, Spain; UK, United Kingdom.
Sweden is not included in this figure as no meticillin-resistant *Staphylococcus aureus* (MRSA) was isolated from the Swedish population in the present study.

DISCUSSION

This study, which included samples from over 32,000 patients who did not have traditional risk factors for antibiotic resistance (an infectious condition at inclusion, recent antibiotic use and recent hospitalization), is the first study that has compared the prevalence and antibiotic resistance of commensal *S. aureus* within and between countries using standardized methods. The use of a central microbiological laboratory to perform the antibiotic resistance analyses excludes interlaboratory bias. The participation of nationwide general practice networks, together with the relatively large study population, leads to accurate estimates of the levels of resistance among commensal

S. aureus in each country in patients visiting general practices for a non-infectious condition.

Only the anterior nares were used as sampling site, which could lead to underestimation of the true prevalence of *S. aureus* and MRSA carriage, because the pharynx, skin and perineum have also been recognized as common habitats of commensal *S. aureus*.[1] However, in order to keep this large surveillance study feasible, we chose to limit the data collection to nasal swabs.

The prevalence of *S. aureus* carriage may have been influenced by interlaboratory differences, although we have limited these by the use of protocols for the isolation and identification of *S. aureus* and the performance of a validation study before the start of this study.[9]

It has been stated that *S. aureus* nasal carriage in healthy adult populations is approximately 27%, although several recent studies have reported a prevalence of around 20%, which is similar to our findings.[1,18,19] In this study, a wide range in nasal *S. aureus* carriage was observed between the participating countries, with participants in Sweden having a prevalence more than twice as high as that of Hungarian participants (29.4% versus 12.1%), even after controlling for age, sex and GP. Whether true differences in *S. aureus* nasal carriage exist between the participating countries is difficult to assess with the current study design, because genetic factors have been found to contribute to the *S. aureus* colonization status of an individual and these were not measured here.[20] However, given the extent of the intercountry variations, future studies on *S. aureus* nasal carriage might explore reasons for these differences.

The prevalence of antibiotic resistance was relatively low among the isolated *S. aureus*, with no resistance being detected for linezolid and vancomycin. In addition, resistance was below 3% for gentamicin and co-trimoxazole, and ciprofloxacin and tetracycline resistance did not exceed 8% in any participating country. However, three isolates were found to be resistant to daptomycin. The restricted use of this agent in clinical practice makes it remarkable that resistance was found among healthy patients in the outpatient setting.[21]

Apart from the well-known high prevalence of resistance to penicillin, the highest resistance and largest inter-country variation was observed for the macrolides. The relatively high extramural use of these agents in Gram-positive infections could be an explanation for this observation.[22] In France and Belgium especially the situation is critical, with azithromycin and erythromycin resistance approaching 20%. With these levels of resistance in commensal *S. aureus*, it can be questioned whether macrolides should still be prescribed empirically for putative *S. aureus* infections in these countries. Except for penicillin and tetracycline, the Swedish commensal *S. aureus* showed the lowest prevalence of resistance to all antibiotic agents tested in comparison with the other participating countries. This was further exemplified by no cases of MRSA being isolated in Sweden and only two *S. aureus* (0.2% of all Swedish *S. aureus* strains) that were multidrug-resistant. The data presented here are, in part, consistent with

data on outpatient antibiotic use from the European Surveillance of Antibiotic Consumption (ESAC), which show high macrolide use in France, for example.[17] However, the high ciprofloxacin resistance found among UK *S. aureus* strains is remarkable, because UK fluoroquinolone use was reported to be the lowest in Europe.[17] Within the APRES project, the antibiotic use in the participating GP networks is currently being investigated and the relationship between use and resistance will possibly explain the results presented here.

The low MRSA prevalence found here, which did not exceed 2.1% in any country, contrasts with the data from *S. aureus* strains causing bloodstream infections, in which the MRSA prevalence ranged from 0.5% in Sweden to 30.2% in Hungary, when only countries participating in APRES are taken into account.[4] The low prevalence of MRSA in healthy humans has been reported previously in several countries.[23,24] The continuing low MRSA prevalence in this population could be the result of the increased public awareness of MRSA and subsequent public health measures taken to control MRSA in recent years.

The population structure of the MRSA strains showed great heterogeneity with 53 unique *spa* types found among the 91 MRSA strains. When these were clustered into *spa*-CCs still much heterogeneity remained, although in a few countries one cluster predominated. In France, more than half of the MRSA belonged to *spa*-CC 008, which corresponds to MRSA CC8, and over 60% of the UK MRSA strains were clustered in *spa*-CC 022/032 – MRSA CC22 (EMRSA-15). Interestingly, *spa* types t008 and t032 were reported, by Grundmann *et al.* who looked at the geographic distribution of dominant invasive *S. aureus* in Europe, to be unexpectedly frequent *spa* types in France and UK, respectively.[25] In the Netherlands, over 50% of the MRSA were grouped in *spa*-CC 011, which included livestock-associated *spa* types.[26] The increase in the proportion of MRSA associated with livestock in the Netherlands had been reported previously.[26,27]

All MRSA strains within *spa*-CC 011 showed resistance to tetracycline, which has been implicated as a good initial marker for MRSA CC398.[28] The high tetracycline use in the veterinary sector could provide a rationale for this association.

The genetic diversity observed here for MRSA strains is in contrast with the clustering of invasive MRSA strains.[25] In a European multicentre study, a convenience sample of 568 *S. aureus* strains (infectious and commensal) isolated over a 10-year period from patients attending a health care centre or hospital, also revealed genotypic diversity.[29] Together with the low resistance found in this study, this implies that most *S. aureus* resistance genes (currently) do not clonally expand or spread easily in the community, but once inside a health care setting are capable of transference from one host to another. This is in contrast with a French study by Dufour *et al.* who reviewed 14 cases with community-acquired MRSA infections in 2002 and concluded that a superadapted MRSA strain appeared to be spreading in the community.[30] To control the levels of MRSA in the extramural setting, continuous surveillance and translation of the results into GP guidelines are of the utmost importance.

In conclusion, among healthy individuals recruited from general practices in nine European countries with different levels and patterns of antibiotic prescription behaviour, a low prevalence of MRSA was found. MRSA strains showed a remarkable genotypic heterogeneity within and between countries, implicating limited spread of MRSA in the community. In addition, the prevalence of *S. aureus* nasal carriage showed a substantial variation by country and the levels of antibiotic resistance were low with the exception of penicillins (all countries) and the macrolides (France, Belgium).

PANEL: RESEARCH IN CONTEXT

Systematic review

We searched PubMed with the search term "*Staphylococcus aureus* AND (healthy OR commensal OR community) AND carriage AND resistance AND Europe" for reports, involving human subjects, published in English before January 3, 2013. This search identified 40 publications. Of these, 23 articles contained data on *S. aureus* prevalence or resistance. The other articles were reviews, studies on the composition of the nasopharyngeal flora, on the interaction of *S. aureus* colonization between animals and humans, or on other staphylococcal species, (exclusively) molecular publications, case reports or small case series.

Interpretation

Of 23 articles on *S. aureus* prevalence and resistance, five were based on samples from patients with specific diseases (e.g. coeliac disease) or within specific health care settings (hospital, long-term care facilities). In three studies, the *S. aureus* strains originated from sites other than the anterior nares, and infectious *S. aureus* strains were the focus in seven studies. One study primarily focused on the carriage status of *S. aureus* without considering the antibiotic resistance pattern of the isolates and two studies reported the prevalence of MRSA carriage only without giving information on the *S. aureus* prevalence. The remaining five studies, conducted in the UK (n=2), Spain, Italy and Portugal, reported both the prevalence of *S. aureus* and MRSA nasal carriage. The prevalence of nasal *S. aureus* carriage ranged from 16% among UK resident adults aged 16+ (n=5,917) in a controlled observational study by Costelloe *et al.*,[31] to 46% among Portuguese high school students (n=107) in a cross-sectional study by Sá-Leão *et al.*[24] The study by Costelloe *et al.* found the highest prevalence of MRSA (3.3%), whereas all other studies reported a MRSA prevalence below 1%. All these five studies had been conducted in a single country and did not exclude patients with recent antibiotic use or hospitalization, thereby potentially affecting the prevalence and resistance within their collection of *S. aureus*.

Our study adds to our knowledge by giving insight in i) the geographic variation concerning the prevalence of commensal *S. aureus* carriage, ii) the presence of antibiotic-resistant *S. aureus* in asymptomatic carriers which is an important reservoir of genetic resistance elements in the community and iii) the molecular epidemiology of asymptomatic MRSA carriage in nine countries in Europe.

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CHAPTER 4

Antibiotic susceptibility of unselected uropathogenic *Escherichia coli* from female Dutch general practice patients: A comparison of two surveys with a 5-year interval

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ABSTRACT

Objectives

To optimize empirical treatment of urinary tract infections (UTIs), regular evaluation of the antibiotic susceptibility of the most common uropathogen, *Escherichia coli*, is necessary. We compared the antibiotic prescription rate for UTIs in women and the *E. coli* antibiotic susceptibility results, including the prevalence of extended-spectrum beta-lactamase (ESBL)-producing strains, in 2009 with data collected 5 years earlier.

Patients and Methods

Urinary samples from female patients with symptoms of uncomplicated UTI in 42 general practices were collected during a 6-month period. Uropathogens were identified and the antibiotic susceptibility of *E. coli* was determined.

Results

We analysed 970 urine cultures, of which 785 (81%) were considered positive (10^3 cfu/mL or more). *E. coli* accounted for 72% of the isolates. ESBLs showed an increase between the two surveys (0.1% versus 1%, $P<0.05$), while no difference in antibiotic susceptibility to the commonly used antimicrobial agents for UTIs was observed. A significantly lower susceptibility rate to co-amoxiclav was observed in the eastern region of the Netherlands, as compared to the northern region (80% versus 92%, $P<0.05$). Consistent with national guidelines, the prescription rate of trimethoprim decreased over time (19% versus 5%, $P<0.05$) whereas nitrofurantoin and fosfomycin rates showed an increase (58% versus 66% and 0% versus 5% respectively, both $P<0.05$).

Conclusions

Over a 5-year period, the antibiotic susceptibility of uropathogenic *E. coli* did not change in female patients with uncomplicated UTI in the Netherlands, but ESBL-prevalence increased. With respect to the prescription of antimicrobial agents, compliance to national UTI guidelines was good.

INTRODUCTION

Acute, uncomplicated urinary tract infections (UTIs) form one of the most common bacterial infections seen in general practice. Especially in adult female patients acute cystitis is frequently diagnosed, with an incidence of 70 per 1000 women each year in the Netherlands.[1]

In addition to instructions for the patient, including among other things sufficient fluid intake and regular emptying of the bladder, the essential element in the treatment of UTIs is antimicrobial drug prescription, which is usually performed on an empirical basis. In the Netherlands the antimicrobial agents of first and second choice for UTIs, according to the guidelines of the Dutch College of General Practitioners (NHG) nitrofurantoin and trimethoprim, accounted for 12% of the total extramural antibiotic use in 2008.[2]

With the high prevalence of UTIs and empirical antibiotic treatment as common practice, there is a substantial risk of the emergence of antimicrobial resistance in uropathogens. Recently, Coque *et al.* described an increased prevalence of extended-spectrum beta-lactamases (ESBLs) in community-acquired infections in Europe, especially among isolates of *Escherichia coli*, which is the uropathogen most commonly isolated.[3]

To control the increasing prevalence of antibiotic resistance, an optimal empirical choice based on current data is essential. These data will be used to update the NHG guideline 'Urinary Tract Infections'. General practitioners (GPs) are requested to comply with these guidelines.

In the last revision of this guideline (2005) nitrofurantoin became the only antimicrobial agent of first choice and trimethoprim changed from first to second place because the antibiotic susceptibility to trimethoprim was decreasing.[4] Fosfomycin was named as the antimicrobial agent in third place.

For the revision in 2005, data from a surveillance study performed by our research group of unselected uropathogenic *E. coli* isolated from GP patients were used.[5-7] General practices (n=21) in the Sentinel Stations project of NIVEL (the Netherlands Institute for Health Services Research) participated in that surveillance study.[8] In 2009 we used the same network of general practices in order to evaluate changes in the antibiotic susceptibility of unselected uropathogenic *E. coli,* and changes in antibiotic use for UTIs, over time. Also the prevalence of ESBL-producing strains was assessed as several studies described an increase in prevalence of ESBLs among *E. coli* isolates.[3,9]

Furthermore, we wanted to determine possible regional differences in the antibiotic susceptibility of the isolated *E. coli*, since these have been reported in community[10] and hospital isolates[11,12] in several other countries.

MATERIALS AND METHODS

Participating GP practices

For the recruitment of patients, general practices (n=42) from the NIVEL Sentinel Stations network participated in this study. This network is nationally representative for age, gender, regional distribution and population density.[8]

In order to assess regional differences, the Netherlands was divided into 4 regions, as described by NIVEL: North (provinces Groningen, Friesland and Drenthe), East (Overijssel, Gelderland and Flevoland), West (Utrecht, Noord- and Zuid-Holland) and South (Zeeland, Noord-Brabant and Limburg).[8]

Patient selection and urine collection

Eligible for inclusion in this study were non-pregnant women aged ≥11 years who consulted their GP practice with symptoms indicating an acute uncomplicated UTI, i.e. strangury, dysuria, urinary frequency and urgency without the presence of fever >38˚C. Exclusion factors were urological or nephrological problems, diabetes mellitus or other immunocompromising diseases. Catheterized patients were also excluded. The period for inclusion was from January 2009 to July 2009.

A short questionnaire was filled in by the GP with questions about the patient's antibiotic use in the past 3 months and whether or not a previous UTI had been diagnosed in the past year. Empirical antimicrobial treatment was recorded when started during the patient's visit.

Finally, a dipslide (Uriline, 56508, Biomérieux, Plainview, NY, USA) was prepared from the urine sample obtained following the instructions of the manufacturer. For incubation and further microbiological analysis the dipslides were sent by mail to the laboratory of Medical Microbiology of the Maastricht University Medical Centre in the Netherlands.

Identification and antimicrobial susceptibility testing of the uropathogens

After the arrival of the dipslides at Maastricht University, bacterial growth was determined and considered positive at $\geq 10^3$ cfu/mL.[1] If no growth was observed on the day of arrival, the assessment of bacterial growth was repeated after an incubation period of 24 hours at 37˚C.

Dipslides showing growth of two or more bacterial species were excluded from the final analysis. For the identification of the uropathogens standard microbiological methods were used.[13]

After isolation and identification, all bacterial strains were kept at -20˚C in peptone/glycerol (30% w/v) for further testing.

The antimicrobial susceptibility of the *E. coli* isolates was determined according to the CLSI criteria[14] using the microdilution method. This involved Mueller-Hinton II cation-adjusted broth (Becton, Dickinson and Company, Sparks, USA), an inoculum of 5 x 10^5 cfu/mL and overnight incubation at 35˚C. The MIC plates with freeze-dried antimicrobial agents were provided by MCS Diagnostics (Swalmen, the Netherlands). The following antimicrobial agents (range in mg/L) were tested: amoxicillin (0.06–128), co-amoxiclav (0.06–128), trimethoprim (0.03–64), co-trimoxazole (0.03–64), norfloxacin (0.03–64), ciprofloxacin (0.003–16) and nitrofurantoin (0.5–512). *E. coli* ATCC 35218 and ATCC 25922 were used as control strains. The breakpoints for susceptibility were in accordance with the EUCAST guidelines.[15]

The susceptibility to fosfomycin was determined by the Kirby-Bauer method (Neo Sensitabs, Rosco Diagnostica, Denmark) and read according to the CLSI guidelines as no EUCAST breakpoints were available for this antimicrobial agent.[14,15]

The *E. coli* isolates resistant to co-amoxiclav were assessed for the presence of ESBL production by means of a combination disc diffusion test (Neo Sensitabs, Rosco Diagnostica, Denmark) with ceftazidime and cefotaxime with and without clavulanic acid, conform the guidelines of the Dutch Society of Medical Microbiology (NVMM).[16] This procedure was also performed on the isolates from the 2004 study.

Comparability of the 2004 and 2009 data

In order to make a reliable comparison, the chosen categories (age, region and uropathogens) and the conditions, including the network of the participating GPs, in the two studies were the same, except for the guidelines used for the *E. coli* susceptibility breakpoints. However, this difference was dealt with by adjusting the original 2004 susceptibility data to the EUCAST breakpoints in the comparison analysis. Because the same research group carried out both studies, the files containing the exact MIC values from the previous study were available and the susceptibility rates of the 2004 data could be calculated according to the EUCAST guidelines.

Statistical methods

For the statistical analysis SPSS 16.0 was used. For the comparison of the antibiotic susceptibility patterns and the antibiotic prescription rates between the two periods the χ^2 test was performed. To assess regional differences the odds ratio (OR) and 95% confidence interval (CI) between the region with the highest susceptibility and the other regions was calculated using a logistic regression model adjusted for age, UTI in the past year and antibiotic use in the past 3 months. A *P*-value <0.05 was considered statistically significant.

Sample size

Our aim at the start of the study was to determine whether the trimethoprim resistance had increased since 2004, and was based on two factors. First, the prescription rate of trimethoprim in the outpatient setting in the Netherlands has decreased in the past years,[2] which could indicate that GPs regularly encounter trimethoprim therapy failure. For this reason, GPs might have decided to prescribe this antimicrobial agent to a lesser extent, irrespective of the revision of the treatment regimen in the NHG guidelines. Secondly, several surveillance studies in the past years have described a decrease in *E. coli* susceptibility to trimethoprim.[5,17] Also Nethmap, the annual report on the use of antimicrobial agents and antimicrobial resistance in the Netherlands based on data from ongoing surveillance systems, reported a decrease to 69% in the *E. coli* susceptibility to trimethoprim among selected urinary strains from outpatient clinics and general practices in 2008.[2]

As the 2004 study described a trimethoprim susceptibility of 77% we estimated a decrease in susceptibility from approximately 80% to 70%. With α=0.05 and β=0.2, ~300 *E. coli* strains were needed to detect this difference. Based on the 2004 study we assumed that 10% of the samples were negative, that 15% showed a mixed culture and that 65% of the positive samples was an *E. coli.*[5] With these figures a total of ~600 samples would be needed for this study. Because we did not want to be dependent on unexpected changes in these figures, we decided to include approximately 1000 samples.

RESULTS

Overall characteristics

A total of 970 urine samples was collected, of which 785 (81%) were considered positive. Of these samples 109 (14%) contained two or more bacteria, with a relatively large proportion in the age group above 70 years: 48 out of 230 samples (21%). The median age of the included female patients was 53 years (range 11–101 years).

No significant difference was observed in the antibiotic use in the past 3 months between patients over 70 years of age, of whom 41% (n=69) used an antimicrobial agent during this time period, versus 35% (n=164) in the 11-70 years age groups (*P*=0.15). Regarding the number of patients with a history of a UTI in the past year, the difference between these two age categories was more obvious: 57% (n=93) in the group above 70 years compared to 42% (n=189) among the patients between 11 and 70 years of age (*P*<0.05).

E. coli was the uropathogen most commonly isolated in all age categories (72%), followed by *Enterococcus faecalis* (6%). *Staphylococcus saprophyticus* was mainly de-

tected in the younger age category (11–20 years), whereas the prevalence of *Klebsiella pneumoniae* increased with increasing age.

Antibiotic use and susceptibility patterns

Empirical antimicrobial treatment was prescribed in 74% (n=719) of the cases. Nitrofurantoin was the antimicrobial agent most frequently prescribed regardless of age (66%). Quinolones and co-amoxiclav were the next most frequently prescribed antimicrobial agents (10% and 8% respectively) and for the former the prescription rate increased with increasing age.

The overall susceptibility of *E. coli* to nitrofurantoin, fosfomycin and fluoroquinolones (ciprofloxacin and norfloxacin) was high (100%, 100% and 97% respectively). *E. coli* showed the lowest susceptibility rate to amoxicillin (66%). No significant differences were observed between the various age groups (Table 1).

Five *E. coli* strains from the 2009 survey were found to be ESBL-positive by the combination disc diffusion test, thus 1% of all *E. coli* isolates.

Table 1. *E. coli* susceptibility rates per age category in 2009

	Age category (years)				Total
	11–20 (n=57)	21–50 (n=154)	51–70 (n=147)	>70 (n=131)	(n=489)
Amoxicillin	61	68	64	70	66
Co-amoxiclav	89	89	85	86	87
Trimethoprim	81	82	77	83	81
Co-trimoxazole	81	86	82	84	84
Norfloxacin	97	98	95	97	97
Ciprofloxacin	98	99	95	97	97
Nitrofurantoin	100	100	99	100	100
Fosfomycin	100	100	99	100	100

Values are given in %.

No differences between the age categories (χ^2 test; P>0.05: range 0.08 (norfloxacin) – 0.41(co-amoxiclav).

Regional differences

When the antimicrobial susceptibilities alone were considered in the statistical analysis, isolates from the eastern region showed statistically more resistance to amoxicillin and co-amoxiclav when compared with isolates from the northern part of the country (OR=2.1, CI: 1.1–4.1 and OR=4.0, CI: 1.4–11.3 respectively). When the variables age, UTI in the past year and antimicrobial agents received in the past 3 months were taken into account, only the co-amoxiclav resistance in the eastern region was significantly

higher (OR=3.9 ,CI: 1.3–11.8) in comparison with the northern region. For the other antimicrobial agents no significant regional differences were found (Table 2).

Table 2. Susceptibility rates to *E. coli* per region in 2009

	Region				Total
	North (n=91)	East (n=94)	West (n=205)	South (n=97)	(n=487)[a]
Amoxicillin	73	57	64	72	66
Co-amoxiclav[b]	92	80	87	90	88
Trimethoprim	84	73	81	86	81
Co-trimoxazole	88	78	83	86	84
Norfloxacin	97	98	96	97	97
Ciprofloxacin	97	99	96	97	97
Nitrofurantoin	100	100	100	99	100
Fosfomycin	100	100	100	100	100

[a] In the case of 2 *E. coli* isolates the region was unknown.
[b] Difference between eastern and northern region calculated with a multivariable analysis including the covariables age, UTIs in the past year and antibiotic use in the past 3 months; OR=3.9, CI: 1.3–11.8).
Values are given in %.

Comparison of 2004-2009 data

Regarding the distribution of the isolated uropathogens between the two surveys, the percentage of *E. coli* increased from 64% to 72% (P<0.05), mainly due to the different *E. coli* rates in the category above 70 years (61% versus 73%, P<0.05). An increase was also observed in *Enterococcus faecalis* from 3% to 6% (P<0.05). On the other hand, the rate of the 'non-fermenters' group decreased from 6% to 3% (P<0.05). This also applied to the group 'Other Gram-negatives' that decreased from 9% to 4% (P<0.05).

The prescription rates of the commonly used antimicrobial agents for UTIs in the 2004 and 2009 surveys are given in Table 3.

The susceptibility of uropathogenic *E. coli* to the most commonly used antimicrobial agents for a UTI did not change over time. In 2004 comparably high *E. coli* susceptibilities were found for nitrofurantoin, fosfomycin and the fluoroquinolones (99%, 99% and 96% respectively). This also applied to amoxicillin (67%), trimethoprim (77%), co-trimoxazole (80%) and co-amoxiclav (88%).

On the other hand, the prevalence of ESBL-producing *E. coli* significantly increased from 0.1% (1/1378) in 2004 to 1% (5/489) in 2009 (P=0.001).

Table 3. Comparison of the UTI prescription rates between the participants of the 2004 and 2009 surveys

	2004 (n=2,047)[a]		2009 (n=719)[a]	
	n	%	n	%
Nitrofurantoin[b]	1,179	58	469	66
Co-amoxiclav[b]	64	3	54	8
Amoxicillin	42	2	13	2
Trimethoprim[c]	387	19	38	5
Co-trimoxazole	62	3	27	4
Fluoroquinolones[c]	290	14	40	10
Fosfomycin[b]	0	0	75	5

UTI, urinary tract infection.

[a] Represents all participants that were prescribed an antimicrobial agent per survey.

[b] Increased prescription rate in 2009 (χ^2 test; $P<0.05$).

[c] Decreased prescription rate in 2009 (χ^2 test; $P<0.05$).

DISCUSSION

No differences were detected in the antibiotic susceptibility of unselected uropathogenic *E. coli* isolates to frequently used antimicrobial agents over a 5-year period in the Netherlands. A similar result has been described in a Belgian study with a 10-year study interval.[18] These results are remarkable, since several European surveillance studies reported a significant increase in resistance among community isolates in the last decade.[9,17]

The ARESC study, an international survey on antimicrobial resistance in uncomplicated UTIs showed a substantial increase in resistance to β-lactams, trimethoprim/sulfamethoxazole, nitrofurantoin and quinolones.[9] However, only 29 *E. coli* isolates were collected in the Netherlands, contributing little to the study outcome. This fact could account for the difference in results from our study, in which 489 *E. coli* strains were examined.

The number of ESBLs, on the other hand, showed a significant increase among our community isolates, with a prevalence of 1% in the present study as compared to 0.1% in 2004. The European Antimicrobial Resistance Surveillance System (EARSS), collecting resistance data from nosocomial isolates throughout Europe, also showed that third-generation cephalosporin resistance in *E. coli* has increased from <1% in 2001 to 5% in 2008 in the Netherlands.[19] In addition, Sturm *et al.* reported recently an ESBL increase from <1 % in 1995 to 2.1% in 2009 among unselected clinical *E. coli* isolates in a Dutch university hospital.[20]

According to these results, the prevalence of ESBL-positive strains seems to be increasing among invasive *E. coli* isolates in this country. In the present study this trend

was also observed in isolates collected in the outpatient setting. This increase in ESBLs highlights the emerging problem of the extended-spectrum beta-lactamases and emphasizes the importance of future surveillance studies to determine whether this trend continues among unselected uropathogenic *E. coli* isolates.

The reason we chose for the assessment of the co-amoxiclav resistant isolates for the detection of the ESBL-positive strains was a practical one, since this antimicrobial agent was already included in the MIC plates used in this survey. However, we did check if this was an adequate method for the detection of all ESBL-positive strains. This was done by checking a database from another project[2] that included selected *E. coli* isolates (n=603) for which the MIC values of co-amoxiclav were available. In this database the MIC values of cefotaxime and ceftazidime, which are the two antimicrobial agents named in most guidelines for the assessment of ESBLs, were also available. We concluded that more than 99% (n=597) of these isolates were assessed correctly according to our approach. We performed the combination disc diffusion test on the 31 *E. coli* isolates that were resistant to ceftazidime or cefotaxime according to the EUCAST guidelines (MIC values of ≥2 mg/L) and observed 15 ESBL-positive strains of which two were co-amoxiclav susceptible. For this reason, we acknowledge that the given ESBL rates could be a small underestimation of the actual rates, but this effect would apply to both years. Therefore, our conclusion on the ESBLs, which is an increase over the period between 2004 and 2009, is still valid as the difference between the two rates found was highly significant.

Looking at the comparability of the 2004 and 2009 surveys, the only difference was the duration of the inclusion period and the sample size. With regard to the inclusion period, participation of GPs was more encouraged in the 2009 study than in 2004, resulting in more practices being involved in the present study (42 versus 21) and less time being needed to collect our data (6 versus 24 months). Considering that in both surveys the participating general practices were part of the NIVEL Sentinel Stations network and chosen in a way that made them representative for the Dutch population, the difference in the number of general practices involved is unlikely to have influenced the comparability of the two surveys. The shorter duration of the 2009 survey should also be considered, since there are studies which describe seasonal peaks in *E. coli* infections with a higher incidence in the summer.[21] As the 2009 study was performed between January and July, both winter and summer were represented in this period, thus diminishing the possible effect of this difference.

The high percentage of positive samples (81%) in comparison with other studies[9,17,22] was possibly due to the low cut-off value (10^3 cfu/mL). Nowadays the Kass criteria (10^5 cfu/mL) are frequently used for the diagnosis of lower UTIs in female patients, but these were originally developed for the diagnosis of acute pyelonephritis and asymptomatic bacteriuria. Several studies have indicated, however, that the lower cut-off value (10^3 cfu/mL) has diagnostic relevance, especially when the patient has clinical symptoms.[23,24] Based on these results, the European urinalysis guidelines rec-

ommended reporting results according to this lower cut-off value.[25] Also other recent studies on the susceptibility of *E. coli* in general practice patients, such as the ECO.SENS study,[17] described their data based on this cut-off value. Finally, the 2004 survey also used the 10^3 cfu/mL cut-off value when describing their results.

The large number of mixed cultures in this study (14%), not observed to this extent in most other studies,[18,26] was mainly influenced by the inclusion of older female patients. The percentage of contaminated cultures was much higher among the included patients aged 70 or over in comparison with the patients between 11 and 70 years of age. Due to a higher co-morbidity, such as urinary incontinence, technical difficulties in obtaining a representative urine sample occur more often in the elderly, resulting in a higher frequency of contamination.[27]

Compared to the 2004 study the higher percentage of *E. coli* among the isolated uropathogens was mainly due to the difference in the group above 70 years. One would expect that the elderly would use more antimicrobial agents and that UTIs would occur more frequently, thereby influencing the distribution of species: fewer susceptible *E. coli* isolates and a wider spectrum of resistant micro-organisms.[28] However, in our study population the percentage of patients that had used antimicrobial agents in the past 3 months was comparable between the elderly (aged >70 years) and the younger age group. This applied to a lesser extent to the patients with a history of UTIs in the past year. Altogether, it appears that the included patients aged over 70 were relatively healthy, resulting in susceptibility rates similar to those in the other age groups and a higher percentage of *E. coli* isolates in this age category in comparison with the 2004 data. The relatively healthy population above 70 years of age in this study could account for these observations, but this could also be a representative sample of the elderly with uncomplicated UTIs, as similar results were described by Grover *et al.*[29] They compared the distribution of uropathogens and *E. coli* susceptibility results to co-trimoxazole, the first-choice drug for UTIs in the United States, between 3 UTI groups: younger uncomplicated patients, uncomplicated elderly (>65 years) and complicated elderly. They concluded that not age, but medical complications, changed the distribution of species, and that *E. coli* susceptibility did not significantly differ between the 3 groups. Although their study lacked statistical power, together with our data it can support the antibiotic policy that the treatment of uncomplicated UTIs in the elderly can be similar to the treatment of younger patients, provided adequate renal function has been confirmed. In this way the use of quinolones, an antimicrobial group prescribed more frequently for UTIs in the elderly, can be restricted. This will have a beneficial effect, as prudent use of quinolones has been emphasized regularly in the last decade due to emerging resistance of uropathogenic *E. coli*.[5,6,30] This problem was already highlighted by the Dutch guidelines, which recommended the use of fluoroquinolones only after antibiotic susceptibility testing has been performed and not on an empirical basis.

Our prescription data confirmed that fluoroquinolones were prescribed less frequently in 2009 than in 2004, whereas co-amoxiclav, the other antimicrobial agent prescribed more often for UTIs in the elderly, showed a rise in prescription rate. On the basis of our data even co-amoxiclav should only be prescribed when the preferred antimicrobial agents, nitrofurantoin, trimethoprim and fosfomycin respectively, are rejected due to factors such as allergies, co-morbidity or side effects.

A decrease in the trimethoprim prescription rate, which was also reported by Nethmap,[2] was observed during this 5-year period. In earlier studies a rise in the resistance of uropathogens to trimethoprim had been described and the Dutch guidelines concerning treatment of UTI in general practice had changed trimethoprim from the first to the second place in the choice of drugs, while nitrofurantoin remained the first-choice agent for uncomplicated UTI. Fosfomycin was named as third-choice antimicrobial agent.[4-6,17] The lower prescription rate of trimethoprim and the higher rates of nitrofurantoin and fosfomycin in this study are consistent with the NHG guidelines, as was the decrease in the prescription rate of fluoroquinolones, thus confirming successful implementation of these guidelines. The current resistance data from 2009 still show high trimethoprim resistance. This could lead to further adaptation of the guidelines, placing fosfomycin instead of trimethoprim as second-choice drug for uncomplicated UTIs, with nitrofurantoin remaining the antimicrobial agent of first choice. However, in addition to the susceptibility results, financial consequences and the side effects of the drugs need to be considered before this decision is taken.

The low susceptibility rate of co-amoxiclav (87%) found, considerably lower than in earlier studies performed in the Netherlands, was remarkable.[5,6,17] In the earlier studies the susceptibility data were based on the CLSI criteria,[15] which have higher breakpoints than the EUCAST guidelines used in the present study. After adjusting our data to the criteria defined by CLSI the susceptibility rate of co-amoxiclav rose to 98%, which is more consistent with the earlier reported percentages. For the other antimicrobial agents no differences in the susceptibility percentages were observed when the EUCAST breakpoints were applied to the 2004 data.

To our knowledge this is the first time a regional difference in *E. coli* susceptibility among community UTI isolates has been observed in the Netherlands. Even after correcting for the factors age, antibiotic use in the past 3 months and a history of UTIs in the past year, the isolates from the eastern part of the country showed a significantly lower susceptibility rate for co-amoxiclav than those from the northern region. The relevance and cause of this regional difference and other associated factors, such as recent hospitalization, could be assessed in future studies.

Concluding, over a time period of 5 years no significant changes in the antibiotic susceptibility of unselected uropathogenic *E. coli* were observed in the Netherlands, although the prevalence of ESBL-producing strains showed an increase to 1% in 2009. Changes in prescription rates of antimicrobial agents over the past 5 years were consistent with changes in the Dutch guidelines for UTI, confirming successful implementa-

tion of these guidelines. The choice of the antimicrobial agent for the treatment of uncomplicated UTIs should not solely depend on the age of the patient, although co-morbidity has to be taken into consideration. Regular surveillance studies need to be performed in the Netherlands in order to optimize empirical treatment and control antimicrobial resistance, especially in ESBLs.

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CHAPTER 5

The importance of gender-stratified antibiotic resistance surveillance of unselected uropathogens:

A Dutch nationwide extramural surveillance study

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ABSTRACT

Few studies have been performed on urinary tract infections (UTIs) in men. In the present study, general practitioners (n=42) from the Dutch Sentinel General Practice Network collected urinary samples from 560 male patients (≥18 years) suspected of UTI and recorded prescribed antibiotic treatment. In this way, the antibiotic susceptibility of Gram-negative uropathogens, including extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* could be determined. In addition, *E. coli* susceptibility and antibiotic prescriptions were compared with data from a similar UTI study among women and with data collected 7 years earlier.

Of 367 uropathogens (66%) identified (≥10^3 cfu/mL), most were Gram-negative (83%) and *E. coli* being isolated most frequently (51%). Antibiotic susceptibility to ciprofloxacin, norfloxacin and nitrofurantoin was 94%, 92% and 88%, respectively, whereas co-amoxiclav (76%) and co-trimoxazole (80%) showed lower susceptibilities. One ESBL (0.5%) was found. A significantly higher proportion of female UTIs was caused by *E. coli* compared with men (72% versus 51%, $P<0.05$). *E. coli* susceptibility tended to be lower in men compared with women, although not reaching statistical significance. No changes in *E. coli* susceptibility were observed over time (all $P>0.05$). Co-amoxiclav and nitrofurantoin prescriptions increased over time (11% versus 28% and 16% versus 23% respectively, both $P<0.05$), whereas co-trimoxazole prescriptions decreased (24% versus 14%, $P<0.05$).

In conclusion, given the observed gender differences in uropathogen distribution and (tendency in) *E. coli* antibiotic susceptibility, empirical male UTI treatment options should be based on surveillance studies including men only. When awaiting the culture result is clinically not possible, fluoroquinolones are advised as first-choice antibiotics for male UTIs in Dutch general practices based on current antibiotic susceptibility data. The prevalence of ESBL-producers was low and no differences were observed in antibiotic susceptibility over a 7-year period. In addition, antibiotic prescriptions changed in accordance with national guidelines during this time period.

INTRODUCTION

Most studies of urinary tract infections (UTIs) focus on female patients because of the higher incidence in women than in men. For this reason, most UTI guidelines are based on studies performed among women, despite the obvious genito-urinary differences.[1] Even so, it was estimated that in 2010 128,000 episodes of UTI were experienced by men in the Dutch general practice setting, representing 16 per 1000 male patients.[2]

Several authors have stressed the importance that gender-stratified UTI surveillance studies should be performed, because of observed differences in antimicrobial susceptibility.[3,4] However, these studies were biased by the inclusion of selected isolates, probably after initial treatment failure, leading to an overestimation of resistance.[3,5]

In the Netherlands, regular surveillance studies are performed on unselected urinary isolates of both men and women to evaluate empirical treatment options in general practice, from which the female data have recently been reported.[6] With this data, we were able to assess the actual difference in *E. coli* susceptibility between male and female patients.

Furthermore, the current treatment options for male UTIs could be evaluated as stated in the guidelines of the Dutch College of General Practitioners (NHG). These are based on the presence of symptoms of tissue invasion (i.e. high fever (>38˚C), chills, malaise, flank or perineal pain) with nitrofurantoin as first-choice agent and trimethoprim as second option for men without these symptoms. Male UTI with symptoms of tissue invasion should preferably be treated with co-amoxiclav followed by fluoroquinolones and co-trimoxazole as shared second-choice.[7]

Also, the prevalence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* was assessed, as male gender has been identified as a risk factor for ESBL-producing Enterobacteriaceae.[8]

Finally, differences in antibiotic susceptibility results over time could be determined for men, because a similar UTI surveillance study was performed between January 2003 and December 2004.[9]

METHODS

Patients and antibiotic prescriptions

General practices (n=42) from the Dutch NIVEL Sentinel General Practice Network were used for the recruitment of patients. The patient population of this network is nationally representative by age, gender, region and population density.[10]

From January 2009 to June 2011, GPs included male general practice patients (≥18 years of age) with symptoms of UTI, i.e. dysuria, urinary frequency and/or urgency. Catheterized patients and patients suspected of having a sexually transmitted disease were excluded. With the exception of benign prostate hypertrophy, patients were also excluded when having urological or nephrological co-morbidity, diabetes mellitus or other immunocompromising diseases.

For all eligible patients, GPs filled in a patient form including age, symptoms of tissue invasion (fever (>38˚C), flank pain) (yes/no) and empirical antibiotic treatment prescribed during the patient's visit (yes/no and if yes the prescribed agent was specified).

Urine collection and processing

Patients provided a fresh-voided (midstream) urine sample, which was used to prepare a dipslide (Uriline, 56508, Biomérieux, Plainview, NY, USA) according to the manufacturer's instructions. For incubation and further microbiological analysis, dipslides were sent to the microbiological laboratory of Maastricht University Medical Centre, the Netherlands.

Bacterial growth was determined on the day of arrival and was considered positive at $\geq 10^3$ cfu/mL.[11] If no growth was observed, dipslides were incubated overnight at 37˚C and assessed again. Standard microbiological methods were used for the identification of the uropathogens.[12] When more than one bacterium was cultured, only the predominant one was included in the final analysis. After isolation and identification, all bacterial strains were kept at -20˚C in peptone/glycerol (30% w/v) for further testing.

Antibiotic susceptibility testing

The susceptibility of Gram-negative isolates was determined using the microdilution method for the following antibiotics: amoxicillin, co-amoxiclav, trimethoprim, co-trimoxazole, norfloxacin, ciprofloxacin and nitrofurantoin. *E. coli* ATCC 35218 and ATCC 25922 were used as control strains. Methods and susceptibility breakpoints were in accordance with the EUCAST guidelines.[13]

Putative ESBL-producing *E. coli* strains were selected on the basis of resistance to co-amoxiclav,[6] and confirmed by means of a combination disc diffusion test (Neo Sensitabs, Rosco Diagnostica, Denmark) with ceftazidime, cefepime and cefotaxime with and without clavulanic acid, following the guidelines of the Dutch Society of Medical Microbiology (NVMM).[14]

Comparison of *E. coli* susceptibility between men and women

From January 2009 to July 2009, a similar surveillance study among women suspected of UTI was performed.[6] This study was conducted by the same research group as the present study and patients were also recruited by GPs from the Dutch NIVEL Sentinel General Practice Network.

Comparison between 2004 and 2011 data

The chosen categories (uropathogens and antibiotics) and all methods, including the network of the participating GPs, were the same in both studies, except for the guidelines used for the *E. coli* susceptibility breakpoints, i.e. CLSI (2004) and EUCAST (2011). Since both studies were performed by the same research group, the original MIC values from the 2004 study were available and 2004 susceptibility data could be recalculated according to the EUCAST guidelines.

Statistical methods

For the comparison of two groups, a Pearson's Chi-square test was used for categorical variables and an independent-samples t-test for continuous variables when parametric assumptions were met. The Mantel-Haenszel extension test for trend was used to determine a trend between more than two categorical variables. The programme PSAW 18.0 for Windows was used for statistical analyses and $P<0.05$ was considered statistically significant.

RESULTS

Patients and culture results

In total, 603 men were included with a median age of 65 years (range 18–97 years). Patients were considered eligible for analyses when data from the patient form were complete (560/603, 93%). The median age of the population with complete data did not differ from the full patient population. Of these 560 patients, 137 (24%) had symptoms of tissue invasion. The median age of the populations with and without signs of tissue invasion was similar (66 years versus 64 years, respectively, $P>0.05$).

A positive culture result was found in 367 (66%) men, of whom 100 (27%) had symptoms of tissue invasion. The probability of a culture being positive increased with age, with men aged 70+ having a 3-times higher chance of a positive culture than men below the age of 50 years (Table 1).

Table 1. Relationship between patient's age and culture result

Age categories (years)	Culture result[a]		OR (95% CI)	*P* for trend[c]
	Bacteriuria[b] (n=367)	No bacteriuria[b] (n=193)		
18-50	58	62	1.0 (reference)	<0.001
51-70	152	87	1.9 (1.2–2.9)	
>70	157	44	3.8 (2.3–6.2)	

OR, odds ratio.
[a] Culture results per age category are given in numbers.
[b] Bacteriuria is defined as the presence of $\geq 10^3$ cfu/mL on the urine dipslide.
[c] P for trend was calculated using the Mantel extension test for trend.

Of all isolated uropathogens, 83% was Gram-negative with *E. coli* being most commonly found (51% of all uropathogens). Differences in uropathogen distribution per age category were not observed (Table 2).

Table 2. Distribution of isolated uropathogens per age category

	Age category (years)			Total
	18–50 (n=58)	51–70 (n=152)	>70 (n=157)	(n=367)
Escherichia coli	57	52	48	51
Proteus mirabilis	3	3	8	5
Klebsiella species	12	5	4	6
Non-fermenters[a]	10	9	11	10
Other Gram-negatives[b]	7	8	15	11
Enterococcus species	3	6	4	5
Other Gram-positives[c]	7	17	10	12

[a] Consist of Pseudomonas and Acinetobacter species.
[b] Consist of Morganella, Citrobacter, Serratia, Pasteurella, Providentia and Enterobacter species.
[c] Consist of *Staphylococcus saprophyticus*, *Staphylococcus aureus* and Streptococcus species
Values are given in %.
No trends with age were observed for the given uropathogens (all *P*>0.05).

Prevalence of antibiotic susceptibility

With respect to the preferred agents for men without signs of tissue invasion according to NHG, Gram-negatives showed a high overall susceptibility to nitrofurantoin (88%), whereas a lower susceptibility was found to trimethoprim (75%). Co-amoxiclav, the first-choice agent for men with signs of tissue invasion according to NHG, showed a relatively low antibiotic susceptibility (76%), as well as co-trimoxazole (80%). On the other hand, the fluoroquinolones (i.e. norfloxacin and ciprofloxacin) had a high susceptibility to the Gram-negatives (92% and 94% respectively) (Table 3). When the Gram-

negative susceptibility data were stratified according to the age categories mentioned earlier, i.e. 18–50, 51–70 and 70+ years, no differences were observed per antibiotic with age (all *P*>0.05, data not shown).

Table 3. Antibiotic susceptibility of Gram-negative uropathogens

	Total (n)	Antibiotic susceptibility (%)						
		AMOX	AMC	TMP	SXT	NOR	CIP	NIT
Escherichia coli	188	65	84	77	78	93	94	100
Proteus mirabilis	18	82	100	82	88	100	100	41
Klebsiella species	21	0	83	94	100	100	100	94
Non-fermenters[a]	38	42	52	35	38	81	84	55
Other Gram-negatives[b]	39	20	49	83	89	89	97	86
All Gram-negative uropathogens	304	53	76	75	80	92	94	88

AMOX, amoxicillin; AMC, co-amoxiclav; TMP, trimethoprim; SXT, co-trimoxazole; NOR, norfloxacin; CIP, ciprofloxacin; NIT, nitrofurantoin.

[a] Consist of Pseudomonas and Acinetobacter species.

[b] Consist of Morganella, Citrobacter, Serratia, Pasteurella, Providentia and Enterobacter species.

Antibiotic use

Empirical antibiotic treatment was prescribed to 359 patients (64%), of whom 284 (79%) were later confirmed as being culture positive. 70+ year old men had a 2-times higher chance to receive antibiotic treatment empirically compared with 18-50 year old men (OR=2.0, 95% confidence interval: 1.2–3.2, *P* for trend <0.001). Fluoroquinolones showed the highest prevalence of prescription (29%), followed by co-amoxiclav (28%), nitrofurantoin (23%) and co-trimoxazole (14%).

In Figure 1 a flow chart is given based on the presence or absence of signs of tissue invasion, empirical therapy and a positive culture result. In the group with signs of tissue invasion (n=137), co-amoxiclav was prescribed more frequently and nitrofurantoin less compared with the prescriptions of the population without signs of tissue invasion (39% versus 24%, *P*=0.004, and 11% versus 27%, *P*=0.002, respectively).

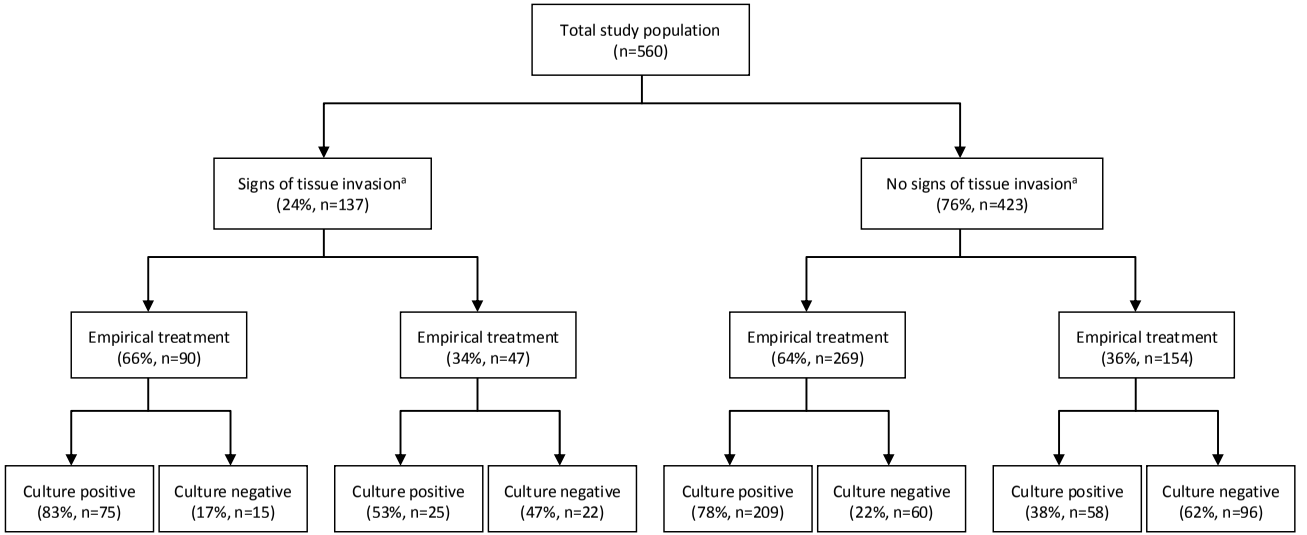

Figure 1. Patient population flow diagram based on signs of tissue invasion, empirical therapy and culture result

[a] Consisted of fever (>38˚C) and flank pain.

[b] Based on the presence of $\geq 10^3$ cfu/mL uropathogens on the urine dipslide.

The denominator of the given % per box was derived from the number given in the box one level up.

Comparison of *E. coli* susceptibility between men and women

In women, *E. coli* accounted for 72% of the total isolates, compared with 51% in men (*P*<0.001). Except for nitrofurantoin, *E. coli* susceptibilities tended to be lower in men compared with women: amoxicillin (65% versus 66%), co-amoxiclav (84% versus 87%), trimethoprim (77% versus 81%), co-trimoxazole (78% versus 84%), fluoroquinolones (94% versus 97%) and nitrofurantoin (both 100%), without being significant for the antibiotics tested (all *P*>0.05).

Comparison of 2004 and 2011 data

No differences were observed between *E. coli* antibiotic susceptibilities in the 2004 and 2011 studies (Table 4). This also applied to the prevalence of extended-spectrum beta-lactamases (ESBLs), as no ESBL-producing *E. coli* were found in 2004 and one (0.5%) in 2011 (*P*=0.44).

Table 4. *Escherichia coli* antibiotic susceptibility between 2004 and 2011 studies according to guidelines used for susceptibility breakpoints

	Study year		
	2004 (n=113)		2011 (n=188)
	CLSI	EUCAST	EUCAST
Amoxicillin	75	72	65
Co-amoxiclav	100	89	84
Trimethoprim	81	78	77
Co-trimoxazole	81	81	78
Norfloxacin	97	95	93
Ciprofloxacin	97	96	94
Nitrofurantoin	97	97	100

Values are given in %.
No significant differences in *E. coli* susceptibilities (EUCAST) were observed between 2004 and 2011 study (all *P*>0.05).

When comparing the antibiotic prescriptions between 2004 and 2011, GPs prescribed more co-amoxiclav and nitrofurantoin (11% versus 28%, *P*<0.001, and 16% versus 23%, *P*=0.023, respectively) and less co-trimoxazole (24% versus 14%, *P*=0.002) in 2011. For fluoroquinolones, amoxicillin and trimethoprim the prevalence of prescription was similar in the two studies (33% versus 29%, 2% versus 3% and 2% versus 1% respectively).

DISCUSSION

In this study of UTIs in Dutch general practice, differences were observed between men and women regarding the distribution of uropathogens, and uropathogenic *E. coli* susceptibilities tended to be lower among men compared with women. For male UTIs, fluoroquinolones showed the highest prevalence of susceptibility to isolated Gram-negative uropathogens, followed by nitrofurantoin. ESBL-producing *E. coli* prevalence was low and no differences in *E. coli* susceptibility were observed over a 7-year period.

The important feature of this study is the participation of general practices from the NIVEL Sentinel General Practice Network. This network has a nationally representative patient population, making it possible to generalize our conclusions to the whole Dutch outpatient population. Moreover, to our knowledge, no other study has been able to include over 600 unselected urinary samples from men suspected of UTI in an outpatient setting.

The fact that no susceptibilities were determined for Gram-positive bacteria could be seen as a limitation. However, empirical treatment for uncomplicated female UTIs is often primarily based on *E. coli* susceptibilities, covering 75-95% of the total spectrum of uropathogens in female UTIs.[15] The proportion of Gram-negatives in the present study is within this range, as also described by Lipski,[16] thereby supporting an evidence-based empirical antibiotic choice for UTI in men.

Also, our study population could be regarded as heterogeneous with the inclusion of men aged 18+, because pathophysiology of male UTI differs with age.[16] We have tried to circumvent this issue by using strict in- and exclusion criteria, in order to obtain a homogeneous sample of men suspected of UTI. Moreover, stratification of antibiotic susceptibility results showed no differences by age.

UTI in men has also been studied by Hummers-Pradier *et al.* and they used 10^2 and 10^5 cfu/mL as cut-off values to determine a UTI.[17] However, their prevalence of UTI (60%), using 10^2 cfu/mL as cut-off, was still lower than our prevalence. The fact that only 36% of the patients included were prescribed an antibiotic in that study and that GPs suspected a UTI in just over 50% of the included patients, suggests that their patient population was at lower risk of UTI than our population with a prescription rate of 64%. In addition, their results were derived from a relatively small patient population (n=79), resulting in estimates with large confidence intervals. Our choice to use 10^3 cfu/mL as cut-off value for the diagnosis of male UTI was based on the European urinalysis guidelines and is supported by Lipsky *et al.*[11,18]

The prevalence of positive culture (66%) we found was also slightly higher than the one reported in the 2004 study (56%).[9] The higher median age in the present study (65 versus 58 years) could explain this difference, because it is known that UTI incidence increases with age, and especially in men, due to the higher frequency of prostatic hypertrophy.[16] In the present study we could confirm this age-dependent trend for positive cultures.

Only 51% of the uropathogens causing male UTI was *E. coli* versus 72% of the UTIs in women. Moreover, uropathogenic *E. coli* susceptibilities tended to be lower in men than in women, although no statistical significance was reached. The difference in these important characteristics for determining optimal empirical treatment, show that men with UTI should be considered as a specific patient category.

Given the more heterogeneous population of uropathogens that causes a UTI in men compared with women, empirical treatment should best be avoided for male UTI. When immediate treatment is necessary based on clinical judgement, our data can be used for the optimal choice of empirical treatment. Our recommendations can be applied to all men aged 18+ suspected of UTI in the Dutch GP setting, because no differences in uropathogen distribution and antibiotic susceptibility per age category were observed.

We found that fluoroquinolones showed a higher prevalence of susceptibility than co-amoxiclav, the current first-choice for men with symptoms of tissue invasion,[7] and the former agents possess specific pharmacokinetic properties leading to high prostate tissue concentrations, which co-amoxiclav lacks.[19-21]

The choice for EUCAST instead of CLSI guidelines had an impact on the *E. coli* susceptibility to co-amoxiclav. Using EUCAST breakpoints, the *E. coli* susceptibility to co-amoxiclav in 2004 decreased from 100% to 89%. The current prevalence of susceptibility of Gram-negatives to co-amoxiclav (77%) is below the proposed threshold (80%) for antibiotics to still be effective as empirical treatment for UTI.[22]

Based on these findings, fluoroquinolones may be regarded as the current preferred treatment in men with tissue invasion. However, in addition to antibiotic resistance data, side effects of the drugs related to age, financial consequences and patient characteristics should be included in the choice of the preferred antibiotics.

Except for *Proteus mirabilis* and non-fermenters, high antibiotic susceptibilities (≥87%) to Gram-negatives were also found for nitrofurantoin. Similar results were described by Guay.[23] Despite this high prevalence of susceptibility, clinical trials on the effectiveness of nitrofurantoin for male UTIs are currently lacking. Since nitrofurantoin does not reach therapeutic concentrations in prostatic tissue,[21] differentiation between cystitis with and without prostatitis is important. So far, no symptoms have been found that allow a conclusive differentiation between these two conditions.[24] The Dutch GP guidelines have based their treatment choices on the presence or absence of so-called 'symptoms of tissue invasion', although no reference was given. Results from future trials would support evidence-based treatment of UTI in men and could help to restrict the use of fluoroquinolones, thereby limiting the chance of the development of antibiotic resistance to this antibiotic group. In this perspective, also the value of fosfomycin for male UTIs needs exploration.[25]

For male UTIs, second-choice agents (i.e. fluoroquinolones) were still prescribed most, although overall compliance with national guidelines improved in comparison with prescription data from 2004. This was shown by an increase in co-amoxiclav and

nitrofurantoin prescriptions, the first-choice agents for men with and without symptoms of tissue invasion respectively.

In the surveillance study on female UTIs in Dutch general practices, *E. coli* susceptibilities from 2004 and 2009 were compared, and also no differences in time trend were observed.[6] Several other studies, including urinary samples from men and women, have reported an increased resistance over time among isolates from an outpatient setting.[26,27] Our regular surveillance studies, the results of which are implemented in clinical practice by including them in the update of the national GP guidelines, appear to have contributed to the control of antibiotic resistance in Dutch general practices. The low prevalence of ESBL-producing *E. coli* is in accordance with these findings.

In conclusion, among unselected *E. coli* originating from Dutch male GP patients suspected of UTI, the prevalence of ESBL-producers was low and no differences were observed in antibiotic susceptibility over a 7-year period. In addition, antibiotic prescriptions changed in accordance with national guidelines during this time period. When culture results can not be awaited, fluoroquinolones may be regarded as the best treatment option for male UTIs in Dutch general practice on the basis of current susceptibility data. Differences in uropathogen distribution, together with the tendency of a lower *E. coli* antibiotic susceptibility in men versus women, make it necessary to base male UTI treatment recommendations on UTI studies performed among men only.

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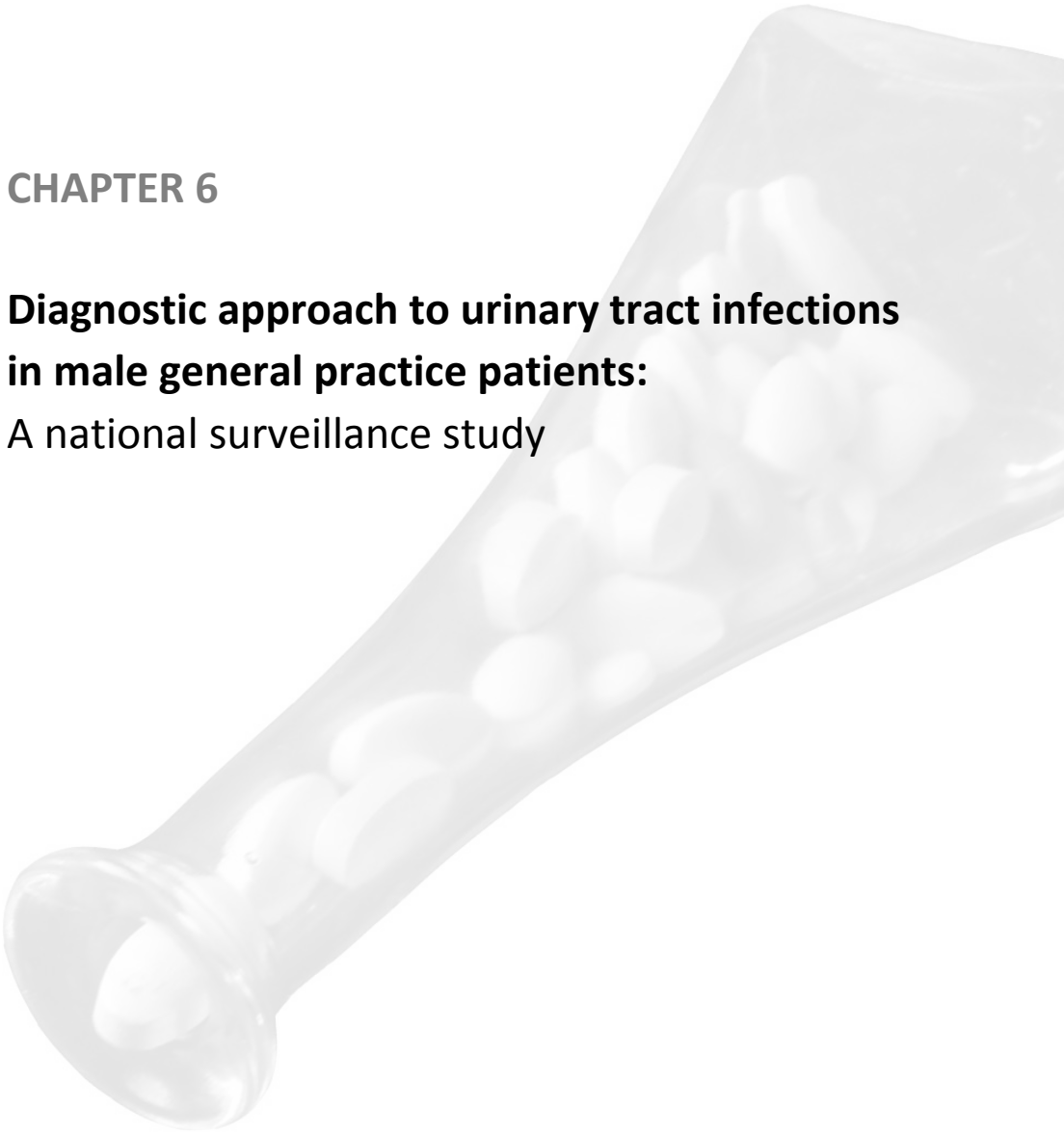

CHAPTER 6

Diagnostic approach to urinary tract infections in male general practice patients: A national surveillance study

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ABSTRACT

Background & aim

Diagnostic urinary tract infection (UTI) studies have primarily been performed among women. We wanted to create a diagnostic algorithm for male general practice (GP) patients suspected of UTI.

Design & Setting

Surveillance study. Dutch Sentinel General Practice Network.

Methods

Clinical information and dipstick results were collected from 603 patients. Algorithm-predicted care was compared with care as usual in terms of sensitivity (antibiotic recommended when UTI was confirmed) and specificity (no antibiotic recommended when no UTI was observed).

Results

Complete information was available from 490/603 (81%) men, of whom 66% (321/490) had a microbiologically confirmed UTI. A diagnostic algorithm recommending antimicrobial prescription in the case of a positive nitrite test or a positive leukocyte esterase test in men aged 60+, had a positive predictive value (PPV) of 83% (95% confidence interval: 78–87) and a negative predictive value (NPV) of 60% (52–66), respectively (area under the ROC curve: 0.78, 0.74–0.82). When both dipstick results were positive in men aged 60+, PPV increased to 90% (83–94), whereas NPV was highest in men ≤60 with negative dipstick results (71%, 59–81).

Sensitivity and specificity of predicted UTI care and usual care did not differ (75% versus 79%, *P*=0.30, and 70% versus 63%, *P*=0.17, respectively).

Conclusions

UTI care provided to Dutch male GP patients is as accurate as predicted care from a diagnostic algorithm. Included clinical information and dipstick tests are useful for ruling in UTI in men, but have limited value in ruling out this diagnosis.

INTRODUCTION

Male urinary tract infections (UTI) are not as frequently observed as female UTIs, although with increasing age this bacterial infection also becomes common in men. In the Netherlands, the average annual incidence rate of male UTIs was 156 per 10,000 compared with 922 per 10,000 women in 2010.[1] At the age of 85, the incidence rate in men was 1319 per 10,000, thus exceeding the average annual incidence rate of female UTIs.

In general practice patients suspected of having a UTI, the evaluation of the predictive value of diagnostic information (i.e. clinical symptoms of UTI and dipstick results) has been performed primarily for women.[2,3] The rationale of these studies is to provide clinicians with a clinical decision rule to improve the quality of UTI care.[4,5] McIsaac *et al.* have shown that the application of a simple diagnostic algorithm can result in the correct treatment of most women with UTI and can reduce unnecessary urine culture testing and antibiotic prescriptions.[5]

Comparable studies of UTI predictors have seldom been performed for male general practice patients. As a consequence, guidelines for diagnosis and treatment of UTI in men are mainly based on female studies, although one can question whether the generalisation of these criteria to men is valid due to the clear genito-urinary differences.[6] A few diagnostic male UTI studies have addressed the predictive value of dipstick test results, but conclusions were contradictory.[6,7] Assessment of clinical symptoms was considered in only one of these studies, based on a relatively small sample size (n=90).[7]

In addition to the development of a diagnostic algorithm, the potential gain in the quality of UTI care to be attributed to the use of the newly developed rule needs to be assessed in clinical practice.[5]

For these reasons, we developed a diagnostic UTI algorithm using clinical information and dipstick results from Dutch male general practice (GP) patients with symptoms indicative of UTI. We also assessed the potential impact of the newly developed diagnostic UTI algorithm by comparing its medical decisions, i.e. prescription of antimicrobial therapy, with usual care.

METHODS

Patients

Dutch general practices (n=42) participating in the NIVEL Sentinel General Practice Network were used for patient recruitment. This network accounts for ~0.8% of the

Dutch population and is nationally representative by age, gender, regional distribution and population density.[1]

From January 2009 to June 2011, GPs included male general practice patients (≥18 years) with symptoms of UTI (dysuria, urinary frequency and/or urgency). Patients having urological or nephrological co-morbidities (with the exception of benign prostate hypertrophy), diabetes mellitus or other immunocompromising diseases were excluded, as well as those catheterized or suspected of having a sexually transmitted disease.

Clinical information and dipsticks

For each eligible patient, the GP filled in a questionnaire giving clinical patient information, i.e. age, history of UTI in the past year, and the presence or absence of dysuria, urinary frequency and/or urgency, fever (>38˚C), and flank pain. These clinical symptoms were derived from the Urinary Tract Infection Guidelines compiled by the Dutch College of General Practitioners (NHG).[8]

All patients provided a fresh-voided midstream urine sample as part of routine clinical practice. The GP or practice assistant used this sample to perform a dipstick test for nitrite and leukocyte esterase (LE) activity. Any change in colour was considered to be a positive test result.[9] Finally, GPs recorded the empirical antimicrobial treatment prescribed. The GPs choice to prescribe an antibiotic (yes/no) empirically was considered to be the 'care as usual'.

Urine culture

From the obtained urine sample, a dipslide (Uriline, 56508, Biomérieux, Plainview, NY, USA) was inoculated for all included men according to the manufacturer's instructions and sent to the department of Medical Microbiology, Maastricht University Medical Centre, the Netherlands. The gold standard to diagnose a microbiologically confirmed UTI in men is uropathogen growth of $\geq 10^3$ cfu/ml.[10] Standard microbiological methods were used for the identification of uropathogens.[11]

Predictors of UTI

Diagnostic algorithms were developed for clinical information and dipstick results separately and combined. Model development was performed according to the procedure described by Little *et al.*, with minor modifications.[13]

In short, to identify relevant predictors of UTI, multivariable logistic regression analyses were carried out from which only the statistically significant predictors ($P<0.05$) were included in the final prediction models. In these analyses, patient's age was dichotomised using the most discriminative cut-off value (60 years of age).

Weights were given to all significant predictors based on the obtained beta coefficients and these weights were used to assign 'weight scores' to all individual patients.

To assess the best performing algorithm, the following parameters were determined for each model, using all possible weight scores as cut-off points: sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively), likelihood ratios for a positive (LR+) and a negative (LR-) test result. Also, the proportion of patients at or above the cut-off value was assessed, which corresponds to the proportion of patients for whom the algorithm would have recommended an empirical antimicrobial agent. Predictive values were most discriminative in the assessment of the best performing algorithm. Receiver operating characteristic (ROC) curves of sensitivity versus one minus specificity were plotted using a web-based calculator.[14]

Potential impact of diagnostic algorithm: comparison of predicted care with usual care

The potential impact of the diagnostic algorithm was determined by comparing the sensitivity and specificity of the algorithm-driven prescription strategy with care as usual.[5] Sensitivity was defined as the proportion of men with a microbiologically confirmed UTI for whom an antimicrobial prescription was recommended. Specificity was defined as the proportion of men without a microbiologically confirmed UTI for whom an antimicrobial prescription was not recommended. A *P*-value <0.05 was considered statistically significant. The programme SPSS 16.0 for Windows was used for statistical analyses.

RESULTS

Patients

In total, 603 male patients participated with a median age of 65 years (range 18–97 years). Clinical information was recorded from 562 of 603 men (93%) and dipstick results were obtained from 87% (527/603) of all included men. Only patients with complete data on clinical information and dipstick results (81%, 490/603) were considered eligible for analysis, of whom 66% (321/490) had a microbiologically confirmed UTI. GPs prescribed an antimicrobial agent to 315 of these 490 male patients (64%).

Influence of missing data

The age of patients with missing data was comparable to the analysed population: median age of 66 years (range: 18–90 years). To assess the influence of missing data, all performed analyses were also done on the sample with either complete clinical

(n=562) or dipstick (n=527) information. In comparison with the presented results, dysuria (OR=1.41, 95% CI: 0.97–2.04) was not predictive for UTI when analyses included all 562 patients with clinical information. For the other predictors, the results did not differ substantially between the sample with complete clinical and dipstick information and those with only complete clinical or dipstick information (data not shown).

Clinical information predictive for UTI

Dysuria (OR=1.57, 1.05–2.36; weight score: 0.5), fever (OR=2.46, 1.22–4.96; weight score: 1.0) and age 60+ (OR=2.49, 1.66–3.74; weight score: 1.0) were found to be predictors for UTI when considering clinical information alone. A weight score of one was the best cut-off value for the prediction model with PPV and NPV of 74% and 51%, respectively (Table 1). This model corresponds to an algorithm recommending antimicrobial prescriptions for men aged 60+ or those with fever. When all three clinical characteristics were present, the predictive value for having UTI was 92%. But patients with none of these three clinical characteristics still had a chance of 43% of having UTI.

Table 1. Clinical weight scores[a] to predict a microbiologically confirmed urinary tract infection[b]

	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])	Correctly classified (%)	LR+ (95% CI)	LR- (95% CI)
≥0 (100%)[c]	100	0	-	-	65.5	1	-
≥0.5 (86.7%)[c],D	91 (88–94)	22 (16–29)	69 (64–73)	57 (44–69)	67.3	1.17 (1.07–1.27)	0.40 (0.27–0.59)
≥1 (66.3%)[c], F/A	75 (70–79)	50 (42–58)	74 (69–79)	51 (43–59)	66.1	1.49 (1.26–1.75)	0.51 (0.42–0.62)
≥1.5 (39.8%)[c], F/A +D	48 (42–53)	75 (68–81)	79 (72–84)	43 (37–49)	57.1	1.92 (1.44–2.55)	0.70 (0.63–0.78)
≥2 (8.2%)[c],F+A	11 (8–15)	96 (92–99)	85 (70–94)	36 (32–41)	40.2	2.98 (1.28–6.96)	0.93 (0.89–0.96)
≥2.5 (4.9%)[c], F+A+D	7 (4–10)	99 (95–100)	92 (72–99)	36 (32–40)	38.6	5.79 (1.38–24.3)	0.94 (0.91–0.97)

PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio for a positive test; LR-, likelihood ratio for a negative test; D, dysuria; F, fever; A, age >60.

[a] Derived from sum of rounded logistic coefficients: dysuria 0.5, fever 1.0 and age >60 years 1.0.

[b] Based on a cut-off value of $\geq 10^3$ cfu/ml.

[c] Clinical weight scores' cut-off point (% at or above the cut-off point).

Dipstick results predictive for UTI

UTI was associated with positive nitrite (OR=8.18, 4.77–14.03; weight score: 2.0) and LE tests (OR=1.80, 1.16–2.80; weight score: 1.0). When the algorithm was based on a weight score of two (recommending prescriptions to patients with a positive nitrite

test), PPV was 90% and NPV 52% (Table 2). In 91% of the cases with both nitrite and LE test results positive, UTI was confirmed. With both tests negative, 40% still had a positive urine culture.

Table 2. Dipstick weight scores[a] to predict a microbiologically confirmed urinary tract infection[b]

	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])	Correctly classified (%)	LR+ (95% CI)	LR- (95% CI)
≥0 (100%)[c]	100	0	-	-	65.5	1	-
≥1 (71.0%)[c],L	82 (78–86)	50 (43–58)	76 (71–80)	60 (51–68)	71.2	1.65 (1.41–1.94)	0.35 (0.28–0.45)
≥2 (66.3%)[c],N	58 (52–63)	88 (82–92)	90 (85–94)	52 (46–58)	68.2	4.87 (3.19–7.43)	0.48 (0.42–0.55)
≥3 (37.3%)[c],L+N	52 (46–57)	90 (84–94)	91 (85–94)	50 (44–55)	64.9	5.14 (3.24–8.17)	0.54 (0.48–0.60)

PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio for a positive test; LR-, likelihood ratio for a negative test; L, leucocyte; N, nitrite.

[a] Derived from sum of rounded logistic coefficients: leucocyte 1.0 and nitrite 2.0.

[b] Based on a cut-off value of $\geq 10^3$ cfu/ml.

[c] Clinical weight scores' cut-off point (% at or above the cut-off point).

Clinical information and dipstick results combined

Age 60+ and positive dipstick results were still predictive for UTI when all diagnostic information was combined in one model (Table 3).

Table 3. Clinical symptoms and dipstick results combined, predictive for UTI[a]

	UTI[a] (n=321)	No UTI[a] (n=169)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Weights[b] in prediction model
Urinary frequency	129 (40)	57 (34)	1.32 (0.90–1.95)	0.83 (0.51–1.34)	-
Dysuria	194 (60)	85 (50)	1.51 (1.04–2.20)	1.35 (0.86–2.11)	-
Urinary urgency	107 (33)	38 (22)	1.72 (1.12–2.65)	1.40 (0.84–2.33)	-
Fever	50 (16)	11 (7)	2.65 (1.34–5.24)	1.58 (0.73–3.39)	-
Flank pain	47 (15)	26 (15)	0.94 (0.56–1.59)	1.42 (0.77–2.63)	-
Age >60 years (0.75)[c]	224 (70)	80 (47)	2.57 (1.75–3.77)	2.11 (1.35–3.31)	1
UTI history in previous year	103 (32)	38 (22)	1.63 (1.06–2.51)	1.03 (0.62–1.71)	-
Nitrite (2.02)[c]	185 (58)	20 (12)	10.13 (6.05–16.99)	7.54 (4.34–13.09)	2
Leucocyte (0.52)[c]	245 (76)	81 (48)	3.50 (2.36–5.21)	1.68 (1.06–2.69)	1

UTI, urinary tract infection; OR, odds ratio.

[a] Based on a cut-off value of $\geq 10^3$ cfu/ml. Numbers are n (%).

[b] Weights are based on the coefficients from multivariable regression analysis.

[c] Coefficients of significant predictors derived from multivariable regression analysis.

When antimicrobial prescription was recommended in the case of a positive nitrite test or a positive LE test in men aged 60+ (representing a weight score of 2), the PPV and NPV were 83% and 60% respectively (Table 4). If both dipstick results were positive in men aged 60+, UTI was microbiologically confirmed in 90% of the cases. UTI was observed in 29% of men not older than 60 with negative dipstick results.

Table 4. Combined dipstick and clinical weight scores[a] to predict a microbiologically confirmed urinary tract infection[b]

	Sensitivity (% [95% CI])	Specificity (% [95% CI])	PPV (% [95% CI])	NPV (% [95% CI])	Correctly classified %	LR+ (95% CI)	LR- (95% CI)
≥0 (100%)[c]	100	0	-	-	65.5	1	-
≥1 (85.3%)[c], L/A	94 (90–96)	30 (24–38)	72 (67–76)	71 (59–81)	71.2	1.34 (1.21–1.48)	0.22 (0.14–0.34)
≥2 (59.6%)[c], N or L+A	75 (70–80)	70 (62–77)	83 (78–87)	60 (52–66)	73.3	2.49 (1.96–3.16)	0.36 (0.29–0.43)
≥3 (40.4%)[c], N+L/A	56 (50–61)	89 (83–93)	90 (85–94)	51 (46–57)	67.1	4.96 (3.21–7.66)	0.50 (0.44–0.56)
≥4 (26.9%)[c], N+L+A	37 (32–43)	92 (87–96)	90 (83–94)	44 (38–49)	56.1	4.82 (2.80–8.28)	0.68 (0.63–0.74)

PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio for a positive test; LR-, likelihood ratio for a negative test; L, leucocyte; A, age >60; N, nitrite.

[a] Derived from sum of rounded logistic coefficients: leucocyte 1.0, age >60 1.0 and nitrite 2.0.

[b] Based on a cut-off value of $\geq 10^3$ cfu/ml.

[c] Clinical weight scores' cut-off point (% at or above the cut-off point).

Potential impact of diagnostic algorithm: predicted care compared with usual care

ROC curve analysis revealed the highest accuracy for the combined model (Figure 1) and this was thus regarded as the best model. The derived algorithm is presented in Figure 2.

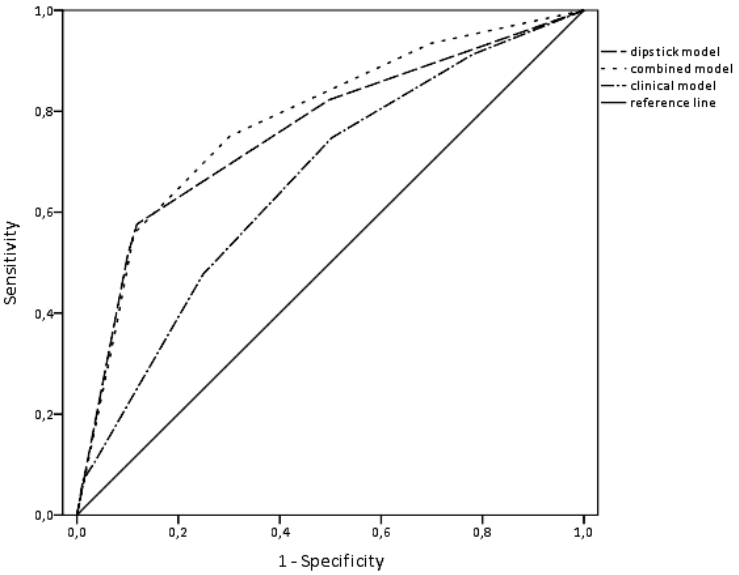

Figure 1. ROC curve analyses of the three UTI prediction models based on clinical symptoms, dipstick results and both together

ROC, receiver operating characteristic; UTI, urinary tract infection.
Area under the ROC curve was 0.66 (95% confidence interval: 0.61–0.71) for the clinical model, 0.76 (0.72–0.80) for the dipstick model and 0.78 (0.74–0.82) for the combined model.

No difference was observed between the proportions of men with UTI for whom antimicrobial prescription was recommended by this algorithm and the care as usual (75% versus 79% respectively, χ^2=1.06, *P*=0.30). The same applied to the proportions of men without UTI for whom antimicrobial prescription was not recommended (70% versus 63% respectively, χ^2=1.91, *P*=0.17).

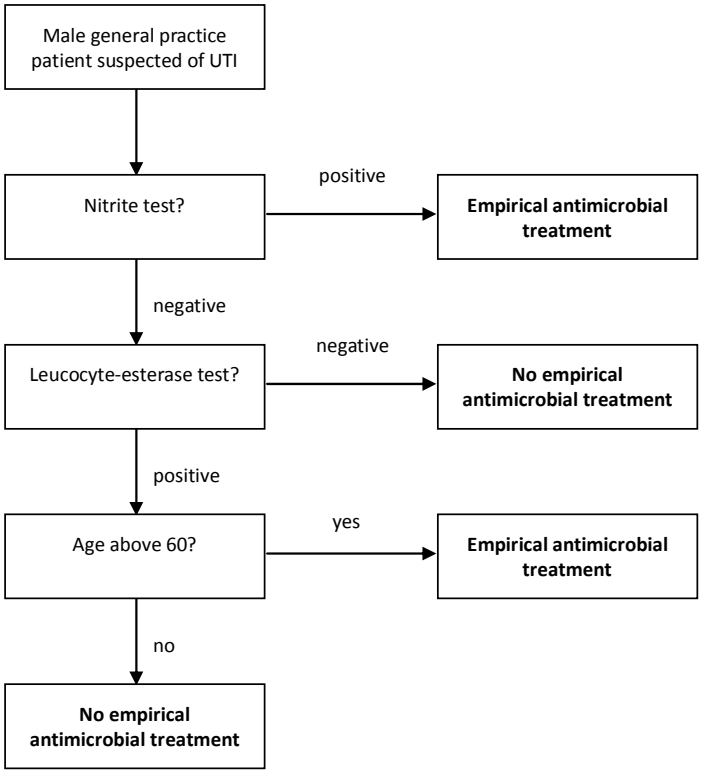

Figure 2. Diagnostic algorithm for male general practice patients suspected of UTI

UTI, urinary tract infection.

DISCUSSION

Summary of main findings

In male general practice patients suspected of UTI, actual care provided by Dutch GPs was as accurate as recommended care based on a diagnostic algorithm. This was assessed by comparing the sensitivities and specificities of both strategies, with the preferred prescription policy based on culture results as the gold standard. Using clinical information and dipstick results, the presence of male UTIs can be predicted correctly.

Strengths and limitations of the study

To the best of our knowledge, this is the first adequately powered study that addressed both dipstick results and clinical information in making a diagnostic algorithm

for UTI in male general practice patients. In addition, the use of a Sentinel General Practice Network, which covers a nationally representative patient population, makes our results applicable to the whole Dutch general practice population.

It has been reported that the severity of symptoms is more predictive for UTI than the actual presence of symptoms.[13] Since no validated questionnaires were available to assess the severity of male UTI symptoms, we decided not to include it in our design. In addition, our recorded diagnostic information was in accordance with the Dutch Urinary Tract Infection guidelines and therefore other information that could have predictive value, like haematuria, was not included in our analyses.[8,15] Although 19% of our data was missing, consequences for the current analyses were limited. Only the predictive value of dysuria for the presence of UTI should be interpreted with caution, as this symptom was not discriminative for UTI when all patients with clinical information available were considered, irrespective of the presence of dipstick data.

Comparison with existing literature

For women suspected of UTI, clinical decisions based on a diagnostic algorithm have been compared with care as usual by McIsaac *et al.*[16] In their algorithm, antimicrobial prescription was recommended when at least two of the following clinical findings were present in women suspected of having a UTI: symptoms for one day, dysuria, positive leukocyte or nitrite test. The number of prescriptions in the case of a negative culture result could be reduced by 40% if their algorithm was implemented. However, this benefit was mainly attributable to the high antimicrobial prescription rate of the participating GPs (89%) as the performance of their algorithm was similar to ours in terms of sensitivity and specificity (80% versus 75% and 54% versus 70%, respectively).

Compared with our results, Hummers-Pradier *et al.* have reported considerably lower positive predictive values for nitrite (90% versus 78%) and LE (76% versus 65%) tests in men.[7] Although UTI prevalence was similar in both settings (65% versus 60%), their patient population included men with co-morbidity, e.g. catheterized patients and men with diabetes mellitus, which could account for the observed differences. Also, their small sample size (n=90) yielded parameters with large confidence intervals. In a 2004 study, using the same network as in the present study and including 422 men, reported PPVs for nitrite (96%) and LE (71%) were similar to ours.[6]

The value of age in predicting UTI in men was earlier reported by Hummers-Pradier, who also observed a positive association between age 60+ and UTI.[7] Although we are aware that a positive urine culture in the elderly must be interpreted with caution because of the relatively high rate of contamination, this is mainly true for the impaired elderly and those who are living in long-term care facilities.[17] In our study population, a relatively healthy, homogeneous patient population was included because of the in- and exclusion criteria used, decreasing the likelihood of contamination.

Implications for research and practice

'Ruling in' UTI diagnosis in men can be done by using dipstick test results, given the high probability of UTI when results, and more specifically the nitrite test, are positive. This supports the previously stated recommendation to start empirical treatment in men suspected of UTI with a positive nitrite test.[6] When dipstick results are unknown, GPs can be quite confident in men older than 60 with dysuria and fever, that a UTI will later be confirmed microbiologically, justifying empirical treatment.

However, 'ruling out' UTI was more problematic, due to the relatively high probability of UTI even when all predictors were absent. Negative dipstick results combined with a relatively low age (≤60) reduce the probability of a UTI compared with negative dipstick results alone (29% versus 40%). However, we consider this chance to still be too high to rule out UTI and therefore opt for urine culture in these patients. Nonetheless, we do acknowledge that our study protocol, with the inclusion of variables predominantly aimed to rule in UTI, will have influenced our results in favour of ruling in a UTI and, consequently, less power to rule out this diagnosis. Therefore, more discriminative diagnostic information for male UTIs is needed and, in particular, information that can rule out UTI. In this perspective, a questionnaire that is sensitive to changes in the symptomatology of UTI in men needs to be developed and tested psychometrically. Also, clinical prediction models need external validation, as the original sample tends to yield results that are too optimistic.[18]

Concluding, the UTI care provided by Dutch GPs to male patients is as good as the care predicted by our diagnostic algorithm, which was based on dipstick test results and the patient's age. Empirical antimicrobial treatment can be initiated in the case of a positive nitrite test or when men above the age of 60 consult with dysuria and fever (>38˚C). For all other men in whom UTI is suspected, GPs should await the urine culture result before starting antimicrobial treatment.

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CHAPTER 7

Predictive value of *Escherichia coli* susceptibility in strains causing asymptomatic bacteriuria for women with recurrent symptomatic urinary tract infections receiving prophylaxis

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ABSTRACT

A significant proportion of women develop a recurrence following an initial urinary tract infection (UTI). In women with recurrent UTI (rUTI), the predictive value of asymptomatic bacteriuria (ASB) for the development of a subsequent UTI has not yet been established and it is not known whether information from an asymptomatic sample is useful in guiding antimicrobial therapy. To address these questions, we used data that originated from the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study: two randomized controlled trials on prevention of rUTI in non-hospitalized pre- and postmenopausal women (n=445).

During 15 months of follow-up, no difference was observed in the time to a subsequent UTI between women with and without ASB at baseline (hazard ratio=1.07, 95% confidence interval: 0.80–1.42). The antimicrobial susceptibility and pulsed-field gel-electrophoresis (PFGE) pattern of 50 *Escherichia coli* strains causing a UTI were compared with that of the ASB strain isolated one month previously. The predictive values of the susceptibility pattern of the ASB strain, based on resistance prevalence at baseline, were ≥76%, except in the case of nitrofurantoin- and amoxicillin-clavulanic acid-resistance. Asymptomatic and symptomatic isolates had similar PFGE patterns in 70% (35/50) of the patients.

In the present study among women with rUTI receiving prophylaxis, ASB was not predictive for the development of a UTI. However, the susceptibility pattern of *E. coli* strains isolated in the month prior to a symptomatic *E. coli* UTI can be used to make informed choices for empirical antibiotic treatment in this patient population.

INTRODUCTION

Approximately 50% of all women will experience a urinary tract infection (UTI) during their lifetime[1] and 27% and 44% develop a recurrence following an initial UTI within 6 months and 1 year respectively.[2,3]

In women who have had at least three episodes of UTI in a period of 12 months, low-dose antibiotic prophylaxis has proved to be effective in reducing the number of recurrences.[4] However, this regimen has the disadvantage that it results in the development of antimicrobial resistance.[5]

In addressing this antimicrobial resistance problem in women with recurrent UTI (rUTI), the information that can be derived from earlier periods of asymptomatic bacteriuria (ASB) has not yet been investigated. ASB is a condition in which uropathogens are isolated from a urinary sample while the patient is not experiencing symptoms indicating a UTI.[6] The relationship between ASB strains and strains that cause a subsequent UTI has been studied in several populations, but has not been addressed in women with rUTI.[7-10] In this population the predictive value of ASB for the development of a subsequent UTI has not been established, nor has the question whether the antibiotic susceptibility pattern of a microorganism isolated from an 'asymptomatic' sample can be used to determine the optimal empirical antimicrobial therapy for a subsequent UTI.

We have therefore investigated, in women with rUTI receiving either antibiotic or non-antibiotic prophylaxis, whether the presence of ASB predisposes to the development of a UTI. Furthermore, we determined whether the *Escherichia coli* ASB strain isolated in the month preceding an *E. coli* UTI was predictive for the strain causing the UTI, by comparing antimicrobial susceptibilities and pulsed-field gel-electrophoresis (PFGE) patterns of the bacteria isolated in both episodes.

MATERIALS AND METHODS

Patients

We used data that originated from the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study.[5,11] Briefly, this study consisted of two randomized controlled multicentre trials comparing 12 months of prophylaxis with cranberries (in premenopausal women) or lactobacilli (in postmenopausal women) with trimethoprim/sulfamethoxazole (TMP/SMX) prophylaxis. Eligible for inclusion were non-hospitalized women over 18 years who had experienced ≥3 symptomatic UTIs in the

year preceding enrolment. Patients were excluded when symptoms of UTI were noted at baseline. Recruitment took place from January 2005 to August 2007.

Midstream urinary samples were collected monthly and when symptoms of UTI were observed during 15 months of follow-up. Dipslides (Uriline, 56508, Biomérieux, Plainview, NY, USA) were prepared from all collected urinary samples and sent to the microbiological laboratory of Maastricht University Medical Centre for identification of the microorganisms and testing of the antimicrobial susceptibility.

If women had a history of functional or structural abnormalities of the urinary tract, of metabolic or hormonal abnormalities, or of impaired host responses, UTIs experienced during follow-up were classified as complicated. UTIs from all other women were classified as uncomplicated. This information was retrieved from the NAPRUTI baseline questionnaire, which also included questions about the number of UTIs in the year preceding enrolment and the sexual activity.

The study protocol was approved by the medical ethics committees of all participating centres, and participants provided written informed consent prior to inclusion.

Predictive value of ASB for the development of UTI

To investigate whether the presence of ASB was predictive for the development of UTI, the participating women were divided into two groups, based on the presence of ASB at baseline. ASB was defined as having $\geq 10^5$ cfu/mL on the baseline dipslide. A UTI was defined as the presence of self-reported symptoms of a UTI together with the isolation of an uropathogen ($\geq 10^3$ cfu/mL).[12] Because not all patients had sent a urine sample when they had symptoms of a UTI, we used occurrence of a clinical recurrence (CR) as secondary endpoint, which was defined as the presence of self-reported symptoms of UTI without microbiological confirmation.[5]

Predictive value of ASB susceptibility and PFGE patterns

Patients were selected in whom the first UTI during the study was caused by *E. coli*. From this group (n=160), the patients were identified in which *E. coli* was detected in the urine specimen collected in the month preceding the first *E. coli* UTI (UTI minus 1 sample, n=76).

Using a random selection procedure, stratified for menopausal status and complicated versus uncomplicated UTI, 50 patients were chosen for the assessment of the antimicrobial susceptibility and PFGE patterns of their *E. coli* isolates. A flow-chart of this selection procedure is given in Figure 1.

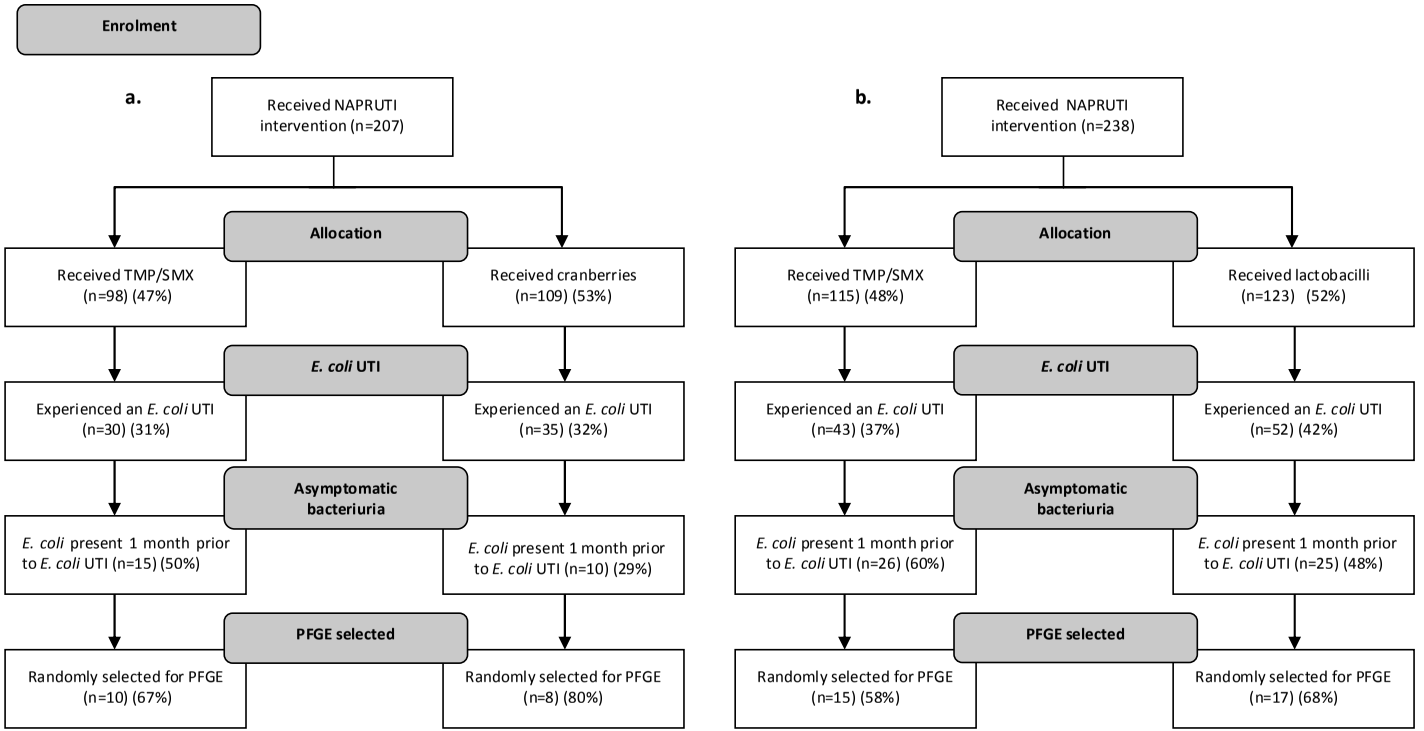

Figure 1. Flow diagram showing the pulsed-field gel-electrophoresis (PFGE) and antimicrobial susceptibility selection procedure for premenopausal women (a) and postmenopausal women (b).

TMP/SMX, trimethoprim/sulfamethoxazole, UTI, urinary tract infection.

Antimicrobial susceptibility testing

The microdilution method used for determining the antimicrobial susceptibility of the *E. coli* isolates involved Mueller-Hinton II cation-adjusted broth (Becton and Dickinson, Company, Sparks, USA), an inoculum of 5 x 10^5 cfu/mL and overnight incubation at 35˚C. The MIC plates with freeze-dried antimicrobial agents were provided by MCS Diagnostics (Swalmen, the Netherlands). Methods and susceptibility breakpoints were in accordance with the EUCAST guidelines.[13]

Pulsed-field gel-electrophoresis

PFGE was carried out using *Xba*I (BioLabs *Inc.*, New England, USA) and interpreted as previously described.[14,15]

Statistical analysis

For the comparison of two groups a Pearson's Chi-square test was used for categorical variables and a Student's t-test or Mann-Whitney U test for continuous variables.

The cumulative probability of being UTI-free during the 15 months' follow-up for women with and without ASB at baseline was studied using Kaplan-Meier estimates, with the logrank test for the comparison. Subsequently, Cox-regression analyses were conducted, which included the following potential confounders: age (in years), number of UTIs in the year preceding enrolment, sexual activity (yes/no), premenopausal status (yes/no), received TMP/SMX prophylaxis (yes/no) and the presence of complicating host factors (yes/no).

To test for potential effect modification by menopausal status or received intervention or uncomplicated versus complicated UTI, interaction terms between these variables and presence of ASB at baseline were included in the statistical models. As these terms were not statistically significant, they were removed from the models and risk estimates were calculated for all women together.

In comparing susceptibilities per antimicrobial agent, asymptomatic and symptomatic strains were considered to be similar when they differed no more than one dilution step. However, isolates were not considered similar when one of them was classified as 'susceptible' and the other as 'resistant'.

For each antimicrobial agent tested, positive and negative predictive values (PPV and NPV respectively) were calculated as described by Vellinga *et al.*[16] Calculated PPVs and NPVs are based on the prevalence of resistance of the *E. coli* strains isolated at baseline. To assess the influence of TMP/SMX prophylaxis, we recalculated our PPVs and NPVs based on the prevalence of resistance of *E. coli* strains isolated after 12 months of TMP/SMX prophylaxis. PPV was the proportion of patients who had an asymptomatic isolate resistant to an antibiotic, in whom the subsequent symptomatic isolate was also resistant to this antibiotic. NPV was the proportion of patients in

whom the asymptomatic isolate was susceptible to an antibiotic and the symptomatic isolate also.

SPSS 16.0 was used for statistical analyses and $P<0.05$ was considered statistically significant. For our power calculation we used the programme STATA, version 12.0.[17,18]

RESULTS

Patients and samples

A total of 207 premenopausal and 238 postmenopausal women participated. The number of ASB and UTI episodes experienced is given in Table 1.

Table 1. Asymptomatic bacteriuria and urinary tract infection episodes

	Premenopausal women (n=207)	Postmenopausal women (n=238)	Total study population (n=445)
ASB[a] at baseline[b]	62 (31)	113 (48)	175 (40)
ASB[a] at any moment during study[c]	170 (82)	219 (92)	389 (87)
UTI[d] at any moment during study[c]	82 (40)	127 (53)	209 (47)

ASB, asymptomatic bacteriuria; UTI, urinary tract infection.

[a] Defined as having $\geq 10^5$ cfu/mL of uropathogens on a dipslide without the presence of self-reported symptoms of UTI.

[b] Baseline dipslides were collected from 433/445 (97%) of the patients: 199/207 (96%) premenopausal and 234/238 (98%) postmenopausal women.

[c] Study duration was 15 months.

[d] Defined as the presence of self-reported symptoms of UTI in combination with the isolation of a uropathogen ($\geq 10^3$ cfu/mL).

Numbers are n (%).

Of 209 patients who experienced a UTI, 160 (77%) had *E. coli* as causative uropathogen. One month preceding these *E. coli* UTIs, *E. coli* was isolated from 76 patients (48%). The mean time interval between the isolation of the included asymptomatic and subsequent symptomatic *E. coli* strain was 15 days (95% confidence interval: 12–17 days). These patients were stratified according to trial (premenopausal (A) versus postmenopausal (B)) and complicated (C) versus uncomplicated (U) status resulting in 4 groups, i.e. AC, AU, BC and BU. Four out of five (80%) AC, 14/20 (70%) AU, 16/26 (62%) BC and 16/25 (64%) BU patients were selected randomly for susceptibility and PGFE analysis (Figure 1a/b).

Predictive value of ASB for the development of a UTI

When comparing the probability of being UTI-free during 15 months of follow-up between women with and without ASB at baseline, no difference was found (43% versus 45%, *P*>0.05) (Figure 2). After inclusion of the confounding factors, ASB at baseline was still not predictive (hazard ratio (HR)=1.07, 95% CI: 0.80–1.42). When CR was used as endpoint, outcomes changed only marginally (data not shown). Considering the number of participants for whom the ASB status was known at baseline (n=433), the proportion of patients without ASB at baseline (60%, 258/433) and the probability of being UTI-free for women without ASB at baseline (45%), we would have been able to detect, at power >80% and α=0.05, a HR of 1.5 for developing a microbiologically confirmed UTI in the follow-up period.

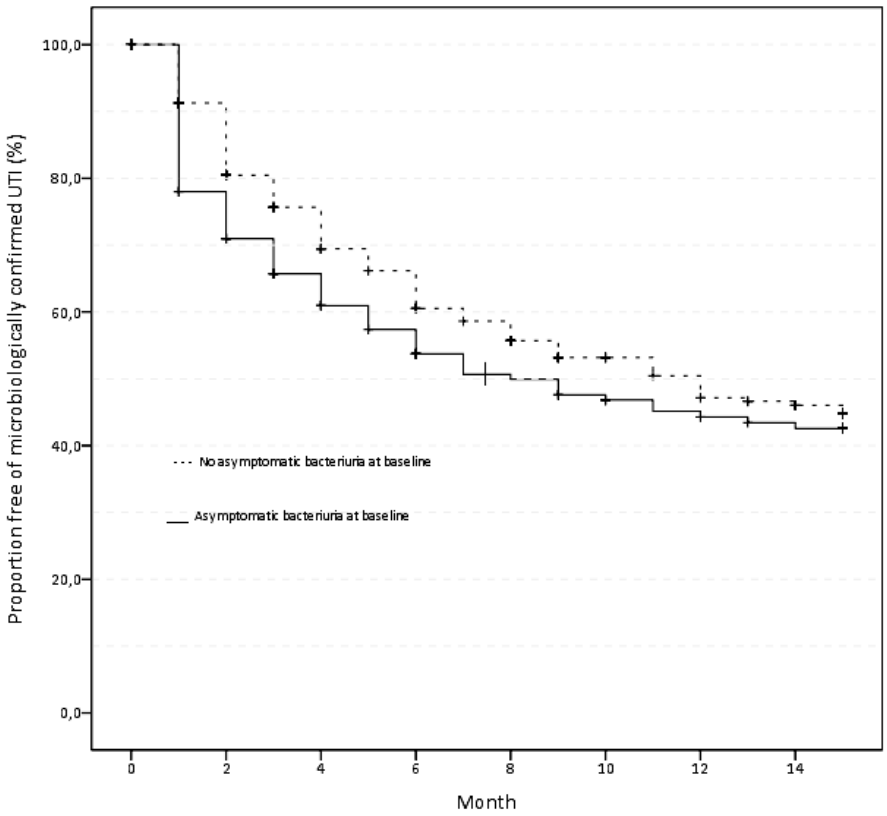

Figure 2. Kaplan-Meier curves for the cumulative probability of being UTI-free during the 15 months of follow-up, for women with and without asymptomatic bacteriuria at baseline

UTI, urinary tract infection.
No difference was observed between the two groups (*P*>0.05).

Predictive value of ASB susceptibility and PFGE patterns

Table 2 gives PPVs and NPVs of the ASB strains. When PPVs and NPVs were based on the prevalence of resistance after 12 months of TMP/SMX prophylaxis, the NPVs of amoxicillin (67%), trimethoprim (24%) and TMP/SMX (24%) decreased due to the development of resistance to these agents. For amoxicillin-clavulanic acid, 7/13 (54%) discordant pairs differed by only one dilution step, but were classified as dissimilar because the asymptomatic *E. coli* was resistant whereas the symptomatic *E. coli* was susceptible. PFGE patterns were similar in 6 of these 7 *E. coli* pairs (86%).

Table 2. Positive and negative predictive value of the resistance pattern of a ASB strain isolated in the month preceding the symptomatic UTI

	Prevalence of resistance (%)[a]	PPV[b] (% [95% CI])	NPV[c] (% [95% CI])
Amoxicillin	35	83 (60–94)	90 (81–95)
Amoxicillin-clavulanic acid	15	34 (21–49)	94 (86–98)
Trimethoprim	34	76 (54–89)	94 (86–98)
TMP/SMX	33	76 (53–89)	94 (86–98)
Norfloxacin	14	100 (96–100)	97 (90–99)
Ciprofloxacin	13	100 (96–100)	97 (90–99)
Nitrofurantoin	0	n.a.[d]	n.a.[d]

ASB, asymptomatic bacteriuria; UTI, urinary tract infection; PPV, positive predictive value; NPV, negative predictive value; TMP/SMX, trimethoprim/sulfamethoxazole.

[a] Prevalence of resistance is based on susceptibility data from *E. coli* strains isolated at baseline.

[b] Proportion of patients who had an asymptomatic isolate resistant to this antibiotic in whom the subsequent symptomatic isolate was also resistant to this antibiotic.

[c] Proportion of patients in whom the asymptomatic isolate was susceptible to this antibiotic and the symptomatic isolate was also susceptible to this antibiotic.

[d] Not applicable because no nitrofurantoin resistant *E. coli* was found at baseline.

From the 50 randomly selected patients, PFGE-concordant *E. coli* isolates were observed in 35 women (70%). Stratification of results into the categories AC, AU, BC and BU showed that, in all groups, over 60% of the *E. coli* pairs were similar (75%, 79%, 63% and 69% respectively, Figure 3). Also, no difference was observed between the prophylactic regimens: 72% (TMP/SMX) versus 68% (non-antibiotics), $P>0.05$.

In two patients with concordant PFGE results, susceptibility patterns differed more than one dilution step for amoxicillin, trimethoprim and TMP/SMX. On the other hand, differing PFGE results were associated with similar susceptibility results in 8 patients.

For patients with concordant PFGE patterns, the median time between the UTI and the UTI minus 1 sample was 12 days (range 1–32). For discordant PFGE patterns, the median time was 20 days (range 5–33, $P<0.05$).

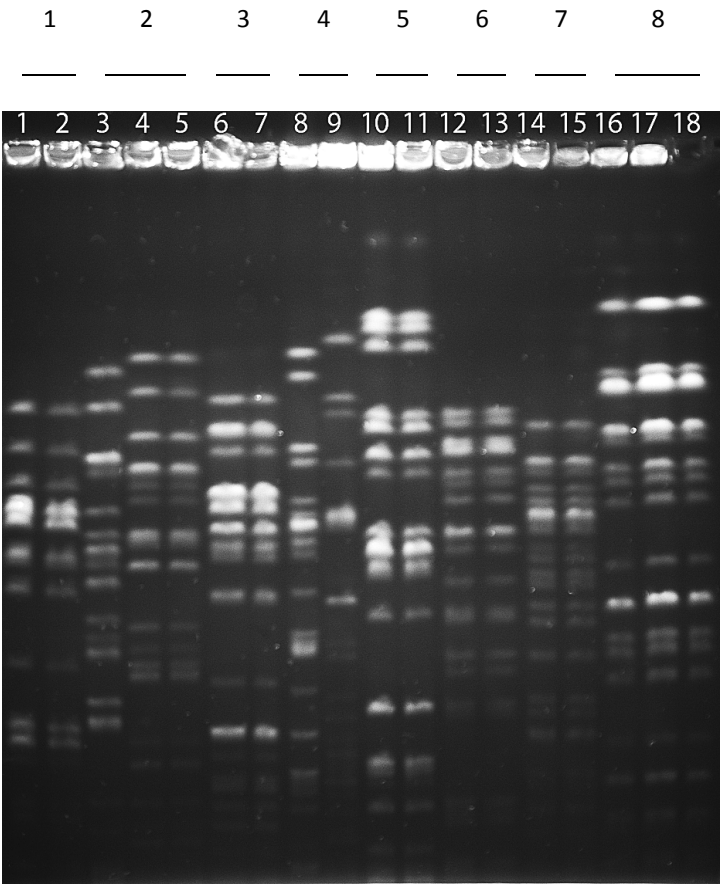

Figure 3. Example of pulsed-field gel-electrophoresis (PFGE) patterns

The patterns of 18 *E. coli* strains, obtained from 8 patients, are shown. Isolates belonging to one patient are indicated by horizontal lines above the PFGE lanes. For each patient, the first lane represents the asymptomatic strain, whereas the second (and third) lane(s) represent(s) the symptomatic strain(s). Two *E. coli* pairs had different PFGE patterns (patients 2 and 4). In patients 2 and 8, PFGE was performed on two symptomatic *E. coli* strains (lanes 4 and 5, and lanes 17 and 18, respectively) with different colony morphology on the blood agar plate, but a similar PFGE pattern.

DISCUSSION

In women with rUTI receiving prophylaxis, the presence of ASB was not found to be a predictor for the development of a UTI during 15 months of follow-up. Nonetheless, antimicrobial susceptibility and the PFGE pattern of asymptomatic *E. coli* strains, iso-

lated within one month before an *E. coli* UTI, correlated well with these characteristics of the UTI-causing strain. At least 90% of the patients had a susceptible *E. coli* UTI strain when a susceptible asymptomatic *E. coli* was isolated, and this applied to all antibiotics tested. Also for resistant asymptomatic isolates, high predictive values (≥75%) were found with the exception of amoxicillin-clavulanic acid (34%). Seventy percent of the *E. coli* pairs had similar PFGE patterns.

The strength of this study was the comparison on an individual level of ASB and UTI strains with regard to antimicrobial susceptibility and PFGE pattern. Furthermore, to the best of our knowledge this was the first study that addressed the predictive value of strains causing ASB in women with rUTI. In addition, this was a subanalysis of data that were acquired prospectively.

A limitation was that in the NAPRUTI study women did not always send a urine sample when experiencing symptoms of a UTI, so the number of microbiologically confirmed UTIs may be underestimated. However, the influence on the present analyses seems limited, as using clinical recurrence as endpoint did not alter our results. Also, our study population could be seen as heterogeneous due to the inclusion of both pre- and postmenopausal women, with differing risk factors and clinical parameters. However, interaction testing between ASB and menopausal status revealed no significant result, indicating that the association between ASB and UTI was similar in both pre- and postmenopausal women. This justifies the combined analysis of these groups in the present study. Furthermore, 40% of all women had ASB at baseline, and almost all experienced an episode of ASB during the study period. Such high levels of ASB have only been observed in specific patient populations, such as catheterized patients and elderly persons in long-term care facilities.[19,20] In the general population, ASB is found in 1-5% of women, and can increase to 8.6% in postmenopausal women.[6,19] The high prevalence in the present study could partly be explained by the use of a single-voided specimen to indicate ASB, instead of two consecutive samples as formulated in the original definition.[20] Nowadays the use of a single specimen has been accepted as a more practical and adequate alternative.[21] Still, it seems that ASB is more common in women with rUTI than in women without such a history. Finally, no placebo group was included in the NAPRUTI study, making our results mainly applicable to women with rUTI receiving prophylaxis. With long-term prophylaxis as the standard treatment for women with rUTI, this limits the external validity of our findings only marginally.

The predictive value of ASB has been previously evaluated in a prospective cohort study, which revealed a marginal association between UTI and ASB (hazard ratio=1.8, 0.9–3.5).[9] Another prospective study among premenopausal women showed that 8% of women with ASB developed a UTI within one week compared with 1% without ASB.[10] However, the women included in these prospective studies had had no history of recurrent UTI, making a comparison with our study difficult. The predictive value of

ASB needs further evaluation in specific subgroups; for example, renal transplant patients, in whom ASB has been associated with a higher incidence of pyelonephritis.[22]

The negative predictive value of ASB strains, in terms of antimicrobial susceptibility, was diminished after long-term TMP/SMX prophylaxis, leading to low predictive values of ASB *E. coli* initially susceptible to amoxicillin, trimethoprim and TMP/SMX (67%, 24% and 24% respectively). The high prevalence of resistance to these agents was expected, since their resistance genes are plasmid linked.[23] However, the clinical relevance of these low negative predictive values is limited, since these agents will not be considered as appropriate treatment options in women receiving TMP/SMX prophylaxis.

The value of antimicrobial susceptibility results from previously obtained isolates has also been studied by Vellinga *et al.*[16] When the interval between two UTIs was no more than three months, they reported NPVs ranging from 78-98% and low PPVs for amoxicillin-clavulanic acid (55%) and nitrofurantoin (20%), similar to our observations. Our observed relatively low PPV for amoxicillin-clavulanic acid was mainly due to the susceptibility breakpoint used, as most discordant *E. coli* pairs differed by only one MIC dilution step. Since all but one of the pairs concerned showed similar PFGE patterns, especially in the case of amoxicillin-clavulanic acid, the use of quantitative susceptibility data is more appropriate than the susceptibility breakpoint. Vellinga explained the low PPV of nitrofurantoin by a relatively quick decay of resistance to this agent when such resistance is found. This is in accordance with the reported low prevalence of resistance of *E. coli* to nitrofurantoin as, for example, shown by the ARESC study in which nitrofurantoin resistance did not exceed 5%.[24] The absence of resistance to nitrofurantoin observed among our *E. coli* is in line with these results. Hence, the recently recorded *E. coli* susceptibility results need to be considered in clinical practice, thereby leading to a more patient-specific approach to the treatment of women with rUTI. This can primarily be applied to patients in the hospital setting, where urinary samples are often requested by treating physicians. General practitioners can apply these findings to patients shortly after hospital discharge.

Of our asymptomatic and symptomatic *E. coli* pairs, 70% had similar PFGE patterns. A similar rate (68%) was found by Russo *et al.*[25] During a 3-month follow-up, Czaja *et al.* reported a same-strain bacteriuria of 7% fourteen days preceding a UTI, that gradually increased to 69% one day before the UTI.[26] Czaja included women aged 18-49 years who had had a history of at least one UTI during the past year, whereas the NAPRUTI inclusion criterion was a minimum of three UTIs. The different patient characteristics of the two study populations might account for the different results.[27] We found a shorter time interval between the two sampling moments for PFGE-concordant *E. coli* strains as compared to PFGE-discordant pairs, which is in accordance with the time-dependent trend reported by Czaja *et al.*[26]

Two *E. coli* pairs had similar PFGE patterns, but differed in susceptibility pattern. This might be due to the presence of integrons encoding for β-lactam, trimethoprim

and sulfamethoxazole resistance, because integrons are too small to be detected by PFGE.[28] PFGE patterns reflect more than the antimicrobial susceptibility alone and this could explain our finding that eight patients had similar antimicrobial susceptibility results but different PFGE patterns.[15]

In conclusion, the presence of ASB is not predictive for the development of UTI in women with rUTI using prophylaxis, but the antimicrobial susceptibility of isolates from asymptomatic urinary samples can be used to guide antibiotic therapy when a UTI develops within one month.

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CHAPTER 8

Determinants of antimicrobial resistance in *Escherichia coli* strains isolated from faeces and urine of women with recurrent urinary tract infections

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ABSTRACT

For women with recurrent urinary tract infections (rUTI), the contribution of antibiotic use versus patient-related factors in determining the presence of antimicrobial resistance in faecal and urinary *Escherichia coli*, obtained from the same patient population, has not been assessed yet. Within the context of the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study, the present study assessed determinants of antimicrobial resistance in *E. coli* isolated from urinary and faecal samples of women with rUTIs collected at baseline. Potential determinants of resistance were retrieved from self-administered questionnaires. From 434 asymptomatic women, 433 urinary and 424 faecal samples were obtained. *E. coli* was isolated from 146 (34%) urinary samples and from 336 (79%) faecal samples, and subsequently tested for antimicrobial susceptibility. Multivariable analysis showed trimethoprim/sulfamethoxazole (TMP/SMX) use three months prior to inclusion to be associated with urine *E. coli* resistance to amoxicillin (odds ratio=3.6, 95% confidence interval: 1.3–9.9), amoxicillin-clavulanic acid (OR=4.4, 1.5–13.3), trimethoprim (OR=3.9, 1.4–10.5) and TMP/SMX (OR=3.2, 1.2–8.5), and with faecal *E. coli* resistance to trimethoprim (OR=2.0, 1.0–3.7). The number of UTIs in the preceding year was correlated with urine *E. coli* resistance to amoxicillin-clavulanic acid (OR=1.11, 1.01–1.22), trimethoprim (OR=1.13, 1.03–1.23) and TMP/SMX (OR=1.10, 1.01–1.19). Age was predictive for faecal *E. coli* resistance to amoxicillin (OR=1.02, 1.00–1.03), norfloxacin and ciprofloxacin (both OR=1.03, 1.01–1.06). In conclusion, in women with rUTI different determinants were found for urinary and faecal *E. coli* resistance. Previous antibiotic use and UTI history were associated with urine *E. coli* resistance and age was a predictor of faecal *E. coli* resistance. These associations could best be explained by cumulative antibiotic use.

INTRODUCTION

The association between antibiotic use and antimicrobial resistance has been convincingly demonstrated.[1-3] On the individual patient level, this is a clinical problem in patients with urinary tract infections (UTI), in particular in women with recurrent UTI (rUTI). The (recurrent) empirical antimicrobial treatment in these women exerts significant resistance pressure on the uropathogens.[4] This pressure also affects the faecal flora, which serves as a resistance reservoir for potential uropathogens.[5,6]

Besides antibiotic use, patient-related factors could be predictive for the presence of resistant uropathogens. For women with rUTI, a higher prevalence of resistance has been observed in women who had complicating host factors compared with women without these factors.[3] Knowledge on the predictors of antimicrobial resistance can be helpful for clinicians to determine the optimal empirical therapy for women with rUTI.

The contribution of antibiotic use versus patient-related factors as determinants of antimicrobial resistance in faecal and urinary *E. coli*, obtained from the same patient population, has not been assessed yet. We recently reported the results of the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study: two studies on antibiotic versus non-antibiotic prophylaxis in pre- and postmenopausal women with rUTI.[2,3] We here investigated the determinants of resistance in the faecal and urinary *E. coli* isolates obtained at baseline in these women.

MATERIALS AND METHODS

Patients

This study was conducted in the context of the 'Non-antibiotic prophylaxis for recurrent urinary tract infections' (NAPRUTI) study.[2,3] The study consisted of two randomized controlled multicentre trials comparing cranberries or lactobacilli with trimethoprim/sulfamethoxazole (TMP/SMX) prophylaxis in pre- and postmenopausal women with recurrent UTIs respectively. Eligible for inclusion were non-hospitalized (both primary care and outpatient clinic) women over 18 years who had experienced three or more symptomatic UTIs in the year preceding enrolment. Patients were excluded when symptoms of UTI were noted at baseline and when any antibiotic had been taken in the previous two weeks. For the present analysis, women were eligible with a urine and/or faecal sample available at NAPRUTI baseline.

Determinants

A baseline questionnaire was completed by all subjects (n=434), including information on the following potential determinants of antimicrobial resistance: the number of UTI episodes in the previous 12 months, antibiotic use in the previous three months (yes/no and if yes subdivided into 4 groups: trimethoprim or TMP/SMX; amoxicillin or amoxicillin-clavulanic acid; nitrofurantoin; quinolones), age (in years), and the presence or absence of complicating host factors. These factors were defined as having a history of functional or structural abnormalities of the urinary tract (yes/no); diabetes mellitus (yes/no); the use of a urinary catheter (yes/no); or of immunosuppressive medication (yes/no). Women who had at least one complicating host factor were classified as having complicating host factors; the remaining women as having no complicating host factors.

Urinary and faecal isolates

Midstream urinary and faecal samples were collected at study baseline. Dipslides (Uriline, 56508, Biomérieux, Plainview, NY, USA) were prepared from collected urinary samples and sent, together with the faecal samples, to the microbiological laboratory of Maastricht University Medical Centre for identification of the microorganisms and antimicrobial susceptibility testing. Bacteriological analysis on faecal samples was done as previously described.[7] Only the predominant *E. coli* strain of each sample was included in the final analysis.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the *E. coli* isolates was determined in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using the microdilution method.[8] The testing included the following agents: amoxicillin, amoxicillin-clavulanic acid, trimethoprim, trimethoprim/sulfamethoxazole (TMP/SMX), norfloxacin, ciprofloxacin and nitrofurantoin.

Statistical analysis

For each antimicrobial agent for which the *E. coli* susceptibility was tested, determinants of antimicrobial resistance were analysed. Crude and adjusted odds ratios (ORs) were calculated for the association between antimicrobial resistance and each determinant. Age was considered as a continuous variable. For the calculation of ORs of the association between antimicrobial resistance and (specific) antimicrobial use, women who had not taken any antimicrobial agent in the previous three months were used as control group. For the calculation of the adjusted ORs, all determinants were included

in a logistic regression model. SPSS 16.0 was used for statistical analyses and a *P*-value of <0.05 was considered statistically significant.

RESULTS

Patients

In total, 433 urinary and 424 faecal samples were obtained from 434 women. 279/433 (64%) urinary samples yielded a uropathogen, of which 146 (52%) were *E. coli*. From 336 faecal samples *E. coli* was isolated (79%). The baseline characteristics of the women from whom an *E. coli* strain was isolated are given in Table 1.

Table 1. Baseline characteristics of women from whom an *Escherichia coli* strain was isolated, stratified for the origin of the sample

	Sample in which *E. coli* strain was isolated		Total study population
	Urine (n=146)	Faeces (n=336)	(n=434)
Age, mean (SD)	52.2 (17.1)	49.9 (17.4)	50.8 (17.4)
Number of UTIs in year preceding enrolment, mean (SD)	7.0 (4.5)	6.8 (3.8)	6.8 (3.9)
Presence of complicating host factors	49 (33.6)	91 (27.2)	126 (29.0)
Postmenopausal	82 (56.2)	172 (51.3)	233 (53.7)
Antibiotics used in previous three months:			
- Any	115 (78.8)	255 (76.1)	339 (78.1)
- Amoxicillin or amoxicillin-clavulanic acid	18 (12.3)	32 (9.6)	38 (8.8)
- Trimethoprim or TMP/SMX	22 (15.1)	56 (16.7)	70 (16.1)
- Nitrofurantoin	55 (37.7)	117 (34.9)	151 (34.8)
- Quinolones	22 (15.1)	53 (15.8)	78 (18.0)

SD, standard deviation; TMP/SMX, trimethoprim/sulfamethoxazole.
Numbers are n (%), unless otherwise stated.

Antimicrobial susceptibility

The antimicrobial resistances of the urinary respectively faecal *E. coli* strains were: amoxicillin: 34%/29%, amoxicillin-clavulanic acid: 17%/10%, trimethoprim: 30%/24%, TMP/SMX: 28%/22%, norfloxacin: 14%/9%, ciprofloxacin: 14%/9%, and nitrofurantoin: 0%/1%.

Urinary *E. coli* were significantly more often resistant to amoxicillin-clavulanic acid than faecal *E. coli* (OR=1.90, 95% confidence interval (CI): 1.08–3.32). All other resistance percentages were not significantly different between urinary and faecal isolates.

Because of the low prevalence of resistance to nitrofurantoin, this agent was excluded from further analysis.

Determinants of antimicrobial resistance

The significant associations between *E. coli* resistance and included potential determinants are given in Table 2.

Table 2. Significant determinants of urinary and faecal *Escherichia coli* antimicrobial resistance

	E. coli resistance to	Determinant	Crude OR (95% CI)[a]	Adjusted[b] OR (95% CI)[a]
Urine	Amoxicillin	TMP/SMX use	4.2 (1.3–13.4)	3.6 (1.3–9.9)
	Amoxicillin-clavulanic acid	TMP/SMX use	6.5 (1.5–27.9)	4.4 (1.5–13.3)
		UTI history	1.13 (1.04–1.23)	1.11 (1.01–1.22)
		Complicating host factors	4.7 (1.9–11.8)	4.0 (1.4–11.7)
	Trimethoprim	TMP/SMX use	4.1 (1.3–13.5)	3.9 (1.4–10.5)
		UTI history	1.12 (1.04–1.21)	1.13 (1.03–1.23)
	TMP/SMX	TMP/SMX use	3.4 (1.0–11.2)	3.2 (1.2–8.5)
		UTI history	1.10 (1.00–1.03)	1.10 (1.01–1.19)
Faeces	Amoxicillin	Age	1.01 (1.00–1.03)	1.02 (1.00–1.03)
	Trimethoprim	TMP/SMX use	3.9 (1.6–9.2)	2.0 (1.0–3.7)
	Ciprofloxacin	Age	1.04 (1.01–1.06)	1.03 (1.01–1.06)
	Norfloxacin	Age	1.04 (1.01–1.06)	1.03 (1.01–1.06)

OR, odds ratio; TMP/SMX, trimethoprim/sulfamethoxazole, UTI, urinary tract infection.

[a] Women who had not taken any antimicrobial agent in the previous three months were used as reference group for the calculation of ORs of the association between antimicrobial resistance and (specific) antimicrobial use.

[b] Adjusted for age, history of a urinary tract infection, antibiotic use in the previous three months and presence or absence of complicating host factors.

The use of trimethoprim or TMP/SMX in the previous three months was associated with increased urinary *E. coli* resistance to amoxicillin, amoxicillin-clavulanic acid, trimethoprim and TMP/SMX. Urinary *E. coli* resistance to the latter three agents was also related to the number of UTIs in the year preceding enrolment, and the presence of complicating host factors was predictive for amoxicillin-clavulanic acid resistance.

In faecal *E. coli*, age was positively associated with resistance to amoxicillin and fluoroquinolones and the use of trimethoprim or TMP/SMX was predictive for trimethoprim resistance.

DISCUSSION

In women with rUTI, different determinants were found for antibiotic resistance in urinary and faecal *E. coli* isolates. For urinary *E. coli* resistance, the use of trimethoprim or TMP/SMX and a history of UTI were the most important determinants, whereas patient's age was the most determinative for faecal *E. coli* resistance.

To our knowledge, this is the first study that assessed the contribution of antibiotic use versus patient-related factors as determinants of antimicrobial resistance in *E. coli* isolated from both urinary and faecal samples, for women with rUTI.

In patients with acute febrile infections, Raum *et al.* have shown that TMP/SMX use influences the prevalence of *E. coli* resistance in faecal samples, even two weeks after cessation of therapy. For beta-lactam antibiotics and doxycycline, this effect was not observed.[5] Likewise, in the present study, in which antimicrobial use had to be stopped two weeks prior to inclusion, *E. coli* resistance was only associated with the use of trimethoprim or TMP/SMX. This suggests that the relationship between antimicrobial use and antimicrobial resistance is stronger for TMP/SMX than for other agents.

In a meta-analysis on the relationship between antibiotic use and resistance, Costelloe *et al.* found weak but detectable associations 12 months after exposure. They argued that this residual effect is likely to be an important driver for the high endemic levels of antimicrobial resistance in the community.[9] The isolated faecal *E. coli* is considered to be the predominant strain of the patient's commensal flora,[10] and is exposed to (orally taken) antimicrobial agents during a patient's lifetime, resulting in gradually increasing resistance with increasing age, as found in the present study.

The specific pathogenesis of rUTI, with increasing evidence of the existence of biofilm-like communities in the bladder from which bacteria are released to cause rUTI, makes resistance of urinary *E. coli* from women with rUTI possibly less dependent on age.[11] In this respect, the lifespan of the biofilm could be of more importance. It has been suggested that the biofilm reduces the susceptibility of *E. coli in vivo* by ineffective antimicrobial diffusion and alterations in the metabolic state of biofilm-associated strains.[11] This might explain the overall trend of higher resistance in urinary compared with faecal *E. coli* found in this study, being however significant for amoxicillin-clavulanic acid only.

In accordance with Costelloe, we found an association between recent antibiotic exposure and resistance in urine.[9] The increased prevalence of resistance to amoxicillin and amoxicillin-clavulanic acid after TMP/SMX use could be explained by the fact that resistance genes for these antimicrobials are located on the same plasmid.[12]

Another predictor of urinary antimicrobial resistance, the number of UTI episodes in the previous year, is probably associated with prior antibiotic use, as previously suggested.[13]

Dutch UTI guidelines for general practitioners recommend amoxicillin-clavulanic acid treatment in patients with complicating host factors.[14] The detected association between amoxicillin-clavulanic acid resistance and the presence of complicating host factors might be attributable to the previous use of this antimicrobial agent in patients with these factors.

A limitation of the study was that antimicrobial use in the previous three months was retrieved from self-administered questionnaires as well as the number of UTIs in the year preceding enrolment. This can make these determinants prone to misclassification bias. However, women were blinded for the resistance status of their *E. coli* isolates, making differential misclassification less likely.

A number of stool cultures did not yield *E. coli* (21%). This indicates that in these samples fewer than 300 cfu of *E. coli* (minimum detection level) were present per gram faeces, as reported previously by a study that used the same bacteriological analysis.[7]

In addition, all included women were asymptomatic, which could limit the translation of our results to women with symptomatic UTI. However, we have recently shown that asymptomatic *E. coli* strains are predictive for strains that cause a symptomatic *E. coli* UTI in women with rUTI.[15]

The associations between patient's age and faecal *E. coli* resistance seem marginal, although it needs to be taken into account that age was considered a continuous variable in all analysis. So, with each increase of one year in age, a women with rUTI has a 1.03 times higher chance of having a ciprofloxacin-resistant faecal *E. coli*. This translates to a 6 times higher chance for a 80-year old women compared with a 20-year old, showing the clinical relevance of this observation.

No genotyping has been performed on the *E. coli* isolates in the present study. However, our aim was to provide clinicians the knowledge on predictors of antimicrobial resistance, which could be helpful when treating a women with rUTI empirically. At that moment, clinicians have no information on the genotypic background of the causative uropathogen at their disposal.

Concluding, in women with rUTI, differences were observed in determinants of urinary versus faecal *E. coli* resistance, which could be attributed to the specific pathogenesis of rUTI. The observed determinants of resistance can best be explained by cumulative antibiotic use.

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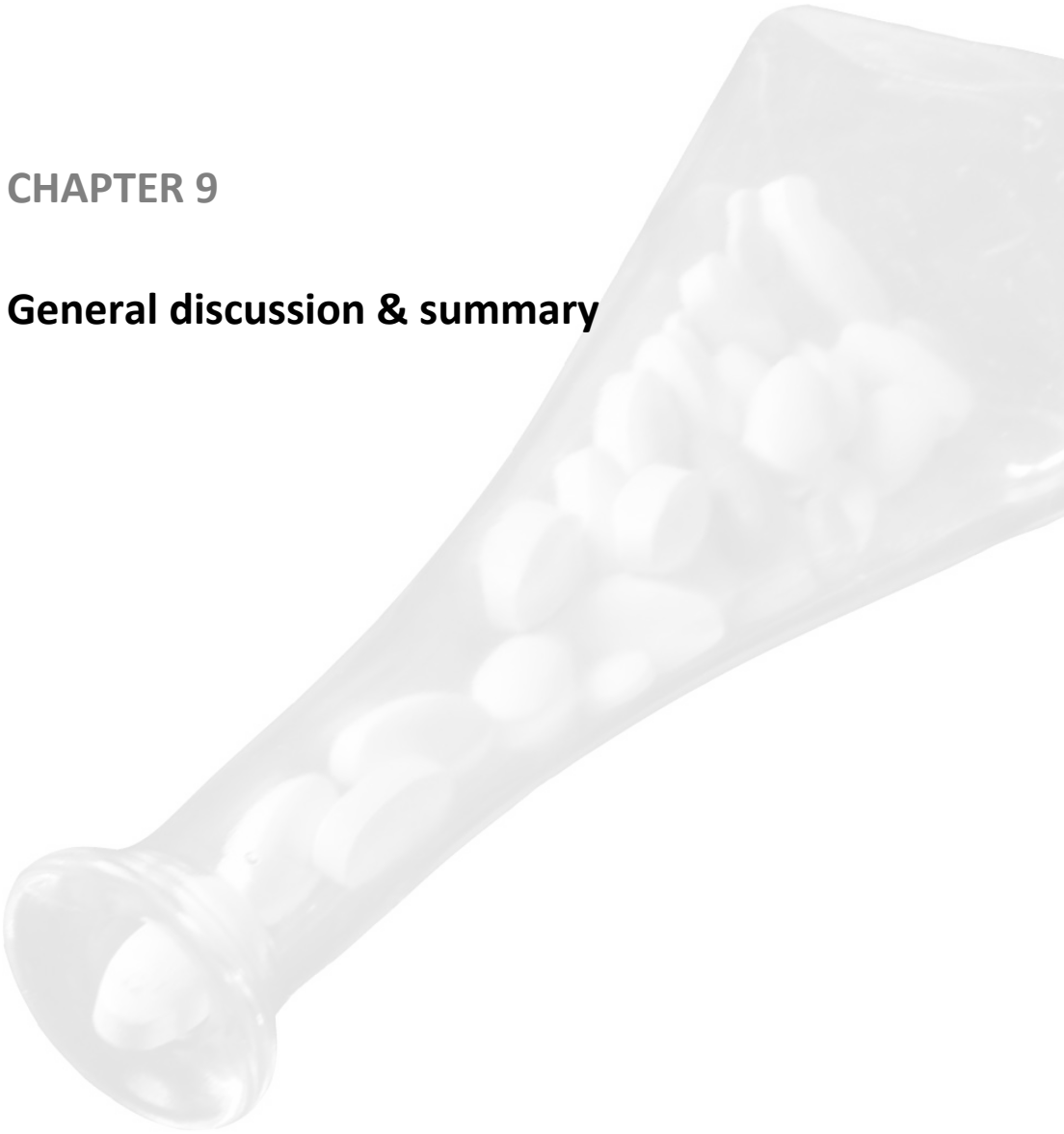

CHAPTER 9

General discussion & summary

The commensal flora is regarded as the most important reservoir of antibiotic resistance.[1] The large number of bacteria, which are exposed to every antibiotic taken during a person's lifetime, gives far more possibilities to acquire resistance than pathogenic bacteria, only present at an infectious site. This results in antibiotic resistance often being present in commensal bacteria first, before disseminating to their pathogenic counterparts.[2] Antibiotic resistance surveillance studies of this reservoir are, therefore, important to act in a timely manner to changing resistance trends and the control of antibiotic resistance at the source.[3]

Another means of controlling antibiotic resistance is to investigate the possible factors that predict the presence of antibiotic resistance. Factors that are found to have predictive value for its presence, can be taken into account by clinicians when deciding upon empirical antibiotic treatment. This will increase the chance of appropriate antibiotic treatment, i.e. an antibiotic prescription to which the causative micro-organism is susceptible.

In addition to an antibiotic prescription being appropriate, it also needs to be necessary. An antibiotic treatment is prescribed unnecessarily, when it is not indicated because the infection is caused by a virus, for example. Clinical decision rules can be helpful to clinicians in determining whether a bacterial infection is present or not, and consequently, whether antibiotic treatment is needed.

In health care, it is strived for treatment to be tailored to the individual patient. Patient-specific treatments will reduce the number of unnecessary and inappropriate antibiotic prescriptions, and thus, can help to reduce the burden of antibiotic resistance. For this reason, the investigation of methods to achieve this goal is of importance.

All the above-mentioned aspects, which can be helpful in controlling antibiotic resistance, have been described in this thesis.

European picture of commensal *Staphylococcus aureus* resistance in the outpatient setting

Most research on antibiotic resistance has focussed on the hospital setting, although over 80% of the antibiotics are being prescribed in primary health care.[4] The consequences of antibiotic use in primary health care on antibiotic resistance levels need to be determined by regular surveillance studies, because the association between antibiotic use and antibiotic resistance has been convincingly demonstrated.[4-6] At present, nosocomial antibiotic resistance data are often translated into GP guidelines for empirical antibiotic treatment, because of the lack of extramural resistance data. This can promote excessive use of broad-spectrum antibiotics in outpatients, which can be prevented by the performance of extramural surveillance studies.[7,8] For this reason, 'The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) study was initiated, of which the set-up of the

study has been described in **Chapter 2**. By investigating the relationship between antibiotic resistance of the commensal nasal flora (i.e. *Staphylococcus aureus* and *Streptococcus pneumoniae*) and the antibiotic use of general practice patients in nine European countries, the appropriateness of prescribing antibiotics was assessed. In other words, this study evaluated the extent to which the antibiotic prescription behaviour was congruent with national community antibiotic resistance patterns. Patients were eligible for inclusion when they visited their GP for a non-infectious condition and had not been prescribed antimicrobial agents nor had been hospitalized in the previous three months. The exclusion criteria are intended to guarantee the participation of "healthy patients" as a proxy for the general population. The participating countries were chosen based on differing levels of prescription behaviour, to maximize the analysis range. The results of this study can lead to country-specific recommendations for the prescription of the antibiotic agents tested. These recommendations can subsequently be translated into national GP guidelines.

It has been stated that the prevalence of *S. aureus* nasal carriage is approximately 27% in an healthy adult population, although several recent studies have reported a prevalence of around 20%.[9-12] Within the APRES study, which included nasal swabs from over 32,000 patients, the overall prevalence of nasal *S. aureus* carriage was found to be 21.6%.

In this study, it appeared that there was a wide range in nasal *S. aureus* carriage between the participating countries, even after correction for age, sex and GP. Participants in Sweden had a prevalence more than twice as high compared with Hungarian participants (29.4% versus 12.1%) (**Chapter 3**). The observed intercountry variations in prevalence could not be explained with the study design used. It can be argued that genetic host factors play a role in this variation, as these have been found to contribute to *S. aureus* nasal carriage and were not taken into account in APRES. Future studies on *S. aureus* prevalence could explore the explanations for these differences given the extent of the intercountry variations found.[13,14]

In general, antibiotic resistance levels among the isolated *S. aureus* were low. Exceptions were the relatively high macrolide resistance, which exceeded 15% in France and Belgium, and the (well-established) high resistance to penicillin in all countries: range 64.9% (Austria) to 87.1% (Spain). The relatively high extramural use of the macrolides in Gram-positive infections could be an explanation for the high prevalence of resistance found to these agents.[15,16]

In addition, three isolates were found to be resistant to daptomycin. The restricted use of this agent in clinical practice makes it remarkable that resistance was found among relatively healthy patients in the outpatient setting.[17] More research is needed to analyze the spread of daptomycin-resistant *S. aureus* in the community.

The prevalence of MRSA did not exceed 2.1% in any of the participating countries, which contrasts the high prevalence reported among invasive hospital *S. aureus* strains, being over 30% in Hungary, one of the APRES countries.[18] The low prevalence

of MRSA in non-hospitalized patients with no signs of infection has been reported previously in several countries.[19-21] The low MRSA prevalence in this population could be the result of the increased public awareness of MRSA and subsequent public health measures taken to control MRSA over the last years.[22]

When the population structure of the MRSA strains was investigated by means of *spa*-typing, a lot of heterogeneity was observed with 53 unique *spa* types found among the 91 MRSA strains isolated within APRES. This genotypic heterogeneity, together with the low resistance found, implies that most *S. aureus* resistance genes (currently) do not spread easily in the community, but once inside a healthcare setting are capable of transference from one host to another. To control the levels of MRSA in the extramural setting, continuous surveillance and translation of surveillance data into clinical guidelines are of the utmost importance.

Uncomplicated urinary tract infections

Another important reservoir of resistance is the faecal flora, consisting of ~10^{14} microbial cells.[23] Bacteria that cause a urinary tract infection (i.e. the uropathogens) often originate from the intestines and reach the urinary tract by colonisation of the urethra. Urinary tract infections (UTIs) are one of the most common bacterial infections observed in primary care, in particular for women, with an annual incidence of 70 per 1000 women in the Netherlands.[24] The standard treatment regimen for this disease is empirical antibiotic prescription and, together with the high UTI incidence, this provides a high risk for the emergence of antibiotic resistance among uropathogens. Nevertheless, we observed no difference in antibiotic resistance of unselected uropathogenic *Escherichia coli* over a 5-year period in female Dutch general practice patients suspected of an uncomplicated UTI (**Chapter 4**). This is remarkable, because a recent international survey on antibiotic resistance in uncomplicated UTI, in which nine European countries and Brazil participated, showed a significant increase in the resistance to several antibiotic agents.[25,26] The low number of Dutch isolates included (29 out of 2,315 *E. coli* isolates) in this international survey could account for the difference in results from our study.

However, we did observe a ten-fold increase in the prevalence of extended-spectrum beta-lactamases (ESBLs) among the isolated *E. coli*: 0.1% (2004) versus 1% (2009). This is in accordance with reports on Dutch clinical isolates,[27,28] and our results show that this trend is currently also apparent in the outpatient setting. Regular surveillance studies are needed to monitor whether this trend continues among unselected uropathogenic *E. coli*.

Surveillance data are of particular value when the results are translated into clinical guidelines and clinicians adhere to these guidelines. For uncomplicated UTI, it was shown that changes in antibiotic prescription rates between 2004 and 2009 were con-

sistent with the Dutch GP guidelines.[24] This confirms the successful implementation of these guidelines into clinical practice.

Urinary tract infections in men

Many guidelines on UTI are based on studies performed among women, because of the higher incidence of this bacterial infection compared with men.[29] However, several authors have stressed the importance of gender-stratified UTI surveillance studies, because of the observed differences in antibiotic susceptibility.[30,31] In **Chapter 5** we showed that this gender difference was also true for unselected uropathogenic *E. coli*, with susceptibilities which tended to be lower for men compared with women and *E. coli* being significantly more often the causative uropathogen in female UTIs than in UTIs in men. This stresses the importance of male UTI guidelines being based on surveillance studies performed among men only.

In addition, we determined the optimal choice for the treatment of UTIs in male general practice patients. This was found to be the fluoroquinolones, which showed the highest antibiotic susceptibility to the isolated Gram-negative uropathogens.

The second best choice, on the basis of antibiotic susceptibility results, was nitrofurantoin. However, this agent does not reach therapeutic concentrations in prostatic tissue,[32] which makes differentiation between cystitis with and without prostatitis important. So far, no symptoms have been found that allow a conclusive differentiation between these two conditions.[33] Results from future trials on the value of nitrofurantoin in male UTIs would support evidence-based treatment of UTI in men and could help to restrict the use of fluoroquinolones, thereby limiting the chance of the development of antibiotic resistance to this antibiotic group. In this perspective, also the value of fosfomycin for male UTIs needs exploration.[34]

Besides up-to-date treatment guidelines, an accurate diagnosis of a UTI is important in controlling antibiotic resistance. For this reason, a diagnostic algorithm for male UTIs was developed based on the predictive value of diagnostic information (clinical information and dipstick results) (**Chapter 6**). A diagnostic algorithm including age 60+ and positive nitrite and leucocyte-esterase (LE) tests showed the highest accuracy for the presence of a UTI. This algorithm had a sensitivity of 75% and a specificity of 70%, in which the preferred prescription policy based on culture results was taken as the gold standard.[35] However, it appeared that this diagnostic algorithm was not superior to the care as usual provided by the participating Dutch GPs in terms of sensitivity (75% versus 79%, P=0.30) and specificity (70% versus 63%, P=0.17).

Nonetheless, it was shown that male UTIs could be 'ruled in' by the use of diagnostic information, given the high probability (≥90%) of a UTI when results, and more specifically the nitrite test, were positive. This supports the previously stated recommendation to start empirical treatment in men suspected of UTI with a positive nitrite test.[29] On the contrast, 'ruling out' UTI was more problematic, due to the relatively

high probability (29%) of UTI even when all predictors were absent. Therefore, more discriminative diagnostic information for male UTIs is needed and, in particular, information that can rule out UTI.

Women with recurrent urinary tract infections

A significant resistance pressure is exerted on the uropathogens of women with recurrent UTIs (rUTIs), because the current standard treatment regimen for women with at least three episodes of UTI during a period of 12 months is low-dose antibiotic prophylaxis.[36] In order to reduce the proportion of women for whom this prophylactic treatment is indicated, the predictive value of asymptomatic bacteriuria (ASB) for the development of a subsequent symptomatic UTI was investigated for this patient category (**Chapter 7**). We demonstrated that the presence of asymptomatic bacteriuria (ASB) was no predictor for the development of a UTI in women with rUTI (hazard ratio=1.07, 95% confidence interval: 0.80–1.42). Hence, the presence of ASB can not discriminate between the women with rUTI who do and do not benefit from prophylactic treatment. However, the predictive value of the susceptibility pattern of an *E. coli* ASB strain, one month prior to an *E. coli* UTI, was ≥76% for all antibiotics tested, except in the case of nitrofurantoin- and amoxicillin-clavulanic acid-resistance. Similar results have been published by Vellinga *et al.* including *E. coli* isolates from general practice patients (both men and women).[37] In addition, our asymptomatic and symptomatic *E. coli* isolates showed similar pulsed-field gel-electrophoresis (PFGE) patterns in 70% (35/50) of the patients. Hence, *E. coli* susceptibility results, recorded in the preceding month, need to be considered in clinical practice, thereby leading to a more patient-specific approach to the treatment of women with rUTI. This can primarily be applied to patients in the hospital setting, where urinary samples are often requested by physicians in charge. General practitioners can apply these findings to patients shortly after hospital discharge.

In **Chapter 8**, we showed that there were different determinants for antibiotic resistance in urinary and faecal *E. coli* isolates from women with rUTI. Patient's age was the most determinative for faecal *E. coli* resistance, whereas the use of trimethoprim with or without sulfamethoxazole and a history of UTI were the most important determinants for urinary *E. coli* resistance.

The isolated faecal *E. coli* is considered to be the predominant strain of the patient's commensal flora,[38] and is exposed to (orally taken) antibiotic agents during a patient's lifetime, resulting in gradually increasing resistance with increasing age, as found here.

The specific pathogenesis of rUTI, with increasing evidence of the existence of biofilm-like communities in the bladder from which bacteria are released to cause rUTI, makes resistance of urinary *E. coli* from women with rUTI possibly less dependent on age.[39] In this respect, the lifespan of the biofilm could be of more importance. It has

been suggested that the biofilm reduces the susceptibility of *E. coli in vivo* by ineffective antimicrobial diffusion and alterations in the metabolic state of biofilm-associated strains.[39] This might explain the overall trend of higher resistance in urinary compared with faecal *E. coli* found in this study. The association between antibiotic use and resistance in urinary isolates was also described by Costelloe *et al.* in a meta-analysis on the relationship between antibiotic use and resistance.[40]

The other determinant of urinary *E. coli* resistance, a history of UTI, is probably associated with prior antibiotic use, as previously suggested.[41]

The provided knowledge on determinants of antibiotic resistance can be helpful for clinicians when determining the optimal empirical treatment for women with rUTI.

Future research to control antibiotic resistance

Antibiotic resistance has been present soon after the introduction of the first antibiotic agents in clinical practice, but only two decades ago it has been recognized as a serious health care problem by influential international health care organizations.[42,43] The abundance of alarming reports on increasing antibiotic resistance stresses the need for new antibiotic classes. However, pharmaceutical companies show marginal interest in the development of new antibiotic agents, because these agents are less profitable as compared with those for chronic diseases. In this perspective, it is hopeful that the Infectious Diseases Society of America's Bad Bugs, No Drugs task force has launched the 10 X 20 initiative, which aims to develop ten new, safe and effective antibiotics by 2020.[44]

Until the discovery of these new agents, the currently available agents need to be conserved, in which regular surveillance studies on antibiotic resistance, performed in the intra- and extramural setting, have a prominent role. The importance of surveillance studies has been exemplified by the mandatory surveillance of MRSA bacteraemia in the UK.[45] This programme resulted in a 56% reduction in the incidence of this disease nationally between 2004 and 2008. In addition, the obtained surveillance data provided a better insight in the epidemiology of MRSA bacteraemia, which enables the development of better directed approaches to control the spread of this multi-resistant bacterium.

In order to optimally benefit from obtained surveillance data, they should be performed in a gender- and age-stratified manner, because many bacterial infections show different spectra and antibiotic susceptibilities of causative pathogens by gender and age.[30,46]

With respect to the setting in which surveillance studies need to be performed, special attention should be given to nursing homes. The increasing life expectancy of humankind results in a need for more long-term care facilities, such as nursing homes, which often house persons aged 65+ with several co-morbidities. Several reports have

implicated that the antibiotic resistance problem in this setting is similar to that in the hospital and, therefore, timely action is required to control it.[47,48]

With the increased opportunities to travel over long distances, it would also be interesting to evaluate the consequences of international travel on (the spread of) antibiotic resistance.[49]

In addition, research should focus on trials of non-antibiotic alternatives for the treatment of bacterial infections. For UTIs, the value of lactobacilli and cranberries has been evaluated, although results are still inconclusive.[50,51] For the treatment of cutaneous bacterial infections, tea tree oil has recently received attention.[52] The development of vaccines against bacterial species and phage therapy are also interesting alternative treatment options in the control of antibiotic resistance.[53,54]

Finally, antimicrobial stewardship programs have been implemented in a few hospitals, which aim to optimize the use of antimicrobials to control the development of resistance and improve patient outcomes. Evaluations of these programs have reported lower antibiotic use and resistance in the hospitals concerned, which were translated into lower costs and shorter hospital stays.[42,55] With these reported successes, it would be interesting to extrapolate such programs to the community setting. Of course, adjustments should be made to tailor these programs to the needs of primary care physicians, but given the promising results in the hospital setting, this attempt seems worthwhile. Together with the development of tools to accurately diagnose bacterial infections, the development of patient-specific approaches to treat them, the investigation of non-antibiotic alternatives for antibiotic treatment, the performance of surveillance studies and the translation of surveillance data into clinical guidelines, this can help in keeping antibiotics effective in the future.

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SAMENVATTING

De commensale flora wordt gezien als het belangrijkste reservoir voor antibioticaresistentie. De grote aantallen bacteriën die blootgesteld worden aan alle antibiotica die een patiënt binnenkrijgt in zijn/haar leven, zorgen ervoor dat commensale bacteriën veel meer mogelijkheden hebben om resistentie te verwerven dan pathogene, die alleen aanwezig zijn tijdens een infectie. Hierdoor is antibioticaresistentie vaak eerst aanwezig in commensale bacteriën, voordat de resistentie zich verspreidt naar hun pathogene tegenhangers.

Om die reden is inzicht in de antibioticaresistentie van dit reservoir belangrijk voor een tijdige start van acties gericht tegen veranderende trends in resistentiepatronen en het beheersen van de antibioticaresistentie.

In **hoofdstuk 1** wordt een literatuuroverzicht gegeven met betrekking tot de begrippen commensale flora en antibioticaresistentie, waarbij speciaal aandacht is voor de bacteriën *Staphylococcus aureus* en *Escherichia coli*. Tevens wordt ingegaan op de verschillende manieren om antibioticaresistentie beheersbaar te houden.

Tot nu toe heeft het meeste onderzoek naar antibioticaresistentie zich gericht op het ziekenhuis, terwijl meer dan 80% van de antibiotica worden voorgeschreven in de eerstelijns gezondheidszorg. De consequenties van dit hoge antibioticagebruik op de mate van antibioticaresistentie in deze setting, dienen bepaald te worden in regelmatige surveillance studies. Daarom is de APRES ('*The appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance*') studie opgezet, waarvan het studieprotocol staat beschreven in **hoofdstuk 2**. Hierin wordt de relatie onderzocht tussen de antibioticaresistentie van commensale flora in de neus (*Staphylococcus aureus* en *Streptococcus pneumoniae*) en het antibioticagebruik van huisartspatiënten in negen Europese landen. Per deelnemend land kan op deze manier worden vastgesteld of het voorschrijven van antibiotica congruent is met de antibioticaresistentie in de samenleving. Op basis van deze resultaten kunnen land-specifieke aanbevelingen worden gedaan met betrekking tot het voorschrijven van de geteste antibiotica. Naderhand kunnen deze aanbevelingen worden vertaald naar nationale richtlijnen voor de huisartspraktijk.

In de APRES studie zijn uiteindelijk meer dan 32.000 neuswatten afgenomen, waarbij een prevalentie van 21,6% werd gevonden voor *S. aureus* dragerschap. Er bleek een substantiële variatie per land te bestaan, zelfs na correctie voor leeftijd, geslacht en huisarts, met een twee keer zo hoge *S. aureus* prevalentie in de Zweedse populatie, in vergelijking met de Hongaarse (29,4% versus 12,1%) (**hoofdstuk 3**).

In het algemeen was de antibioticaresistentie onder de geïsoleerde *S. aureus* stammen laag, met uitzondering van de macrolide-resistentie in Frankrijk en Belgie (>15%) en resistentie tegen penicilline in alle landen: van 64,9% (Oostenrijk) tot 87,1% (Spanje).

Wanneer de prevalentie van meticilline-resistente *S. aureus* (MRSA) werd berekend op basis van het totaal aantal geïsoleerde *S. aureus* stammen, was deze prevalentie maximaal 2,1% (in België). Tevens werd een hoge mate van genotypische heterogeniteit gezien, met 53 unieke *spa* types onder de 91 geïsoleerde MRSA stammen binnen APRES. Deze genotypische heterogeniteit, samen met de relatief lage resistentie, impliceert dat de meeste *S. aureus* resistentiegenen zich (op dit moment) niet eenvoudig kunnen verspreiden in de samenleving. Echter, om deze lage resistentie te handhaven in de extramurale setting, is continue surveillance en vertaling van deze surveillance data naar klinische richtlijnen van zeer groot belang.

Een ander belangrijk resistentiereservoir is de faecale flora, bestaand uit ~10^{14} microbiële cellen. Bacteriën die een urineweginfectie veroorzaken (de uropathogenen) hebben hun oorsprong meestal in de tractus gastro-intestinalis en bereiken de urinewegen door het coloniseren van de urethra. Urineweginfecties (UWIs) zijn één van de meest voorkomende bacteriële infecties in de huisartspraktijk, voornamelijk bij vrouwen, met een jaarlijkse incidentie van 70 per 1000 Nederlandse vrouwen.

De standaard therapie voor deze aandoening is het empirisch voorschrijven van antibiotica. Samen met de hoge UWI incidentie resulteert dit in een hoog risico op het ontstaan van antibioticaresistentie onder uropathogenen. Desalniettemin konden wij geen verschillen in antibioticaresistentie aantonen over een periode van 5 jaar bij ongeselecteerde uropathogene *E. coli* stammen, verzameld uit de urinemonsters van Nederlandse vrouwelijke huisartspatiënten met de verdenking op een ongecompliceerde UWI (**hoofdstuk 4**). Toch was wel een tienvoudige toename in de prevalentie van *extended-spectrum beta-lactamases* (ESBLs) zichtbaar onder de geïsoleerde *E. coli*: 0,1% (2004) versus 1,0% (2009).

Surveillance data zijn voornamelijk bruikbaar indien de resultaten hiervan vertaald worden naar klinische richtlijnen en als clinici handelen conform deze richtlijnen. Voor ongecompliceerde UWIs zagen we dat de veranderingen in het voorschrijven van antibiotica consistent waren met de Nederlandse huisartsstandaarden. Deze richtlijnen blijken derhalve succesvol te zijn geïmplementeerd in de dagelijkse praktijk.

Veel UWI richtlijnen zijn gebaseerd op studies waarbij alleen vrouwelijke patiënten geïncludeerd zijn, vanwege de hogere UWI incidentie bij vrouwen dan bij mannen. In **hoofdstuk 5** laten we zien dat de antibioticagevoeligheid van uropathogene *E. coli* consistent lager is voor mannen dan voor vrouwen. De verschillen waren echter voor geen van de geteste antibiotica statistisch significant. Wel werden er significante verschillen aangetoond tussen mannen en vrouwen voor wat betreft de bacteriën die een UWI veroorzaakten. *E. coli* werd minder vaak bij mannen dan bij vrouwen aangetroffen

als veroorzaker (51% versus 72%). Dit toont aan dat richtlijnen voor UWIs bij mannen bij voorkeur gebaseerd moeten zijn op surveillance studies met de inclusie van alleen mannelijke patiënten.

Door de grotere diversiteit aan uropathogenen, kan empirische therapie voor deze groep niet worden aanbevolen. Indien empirische therapie klinisch noodzakelijk is, bleek een fluorochinolon de optimale therapie voor een UWI bij een Nederlandse, mannelijke huisartspatiënt te zijn, gebaseerd op de hoogste antibioticagevoeligheid onder de geïsoleerde Gram-negatieve uropathogenen. Nitrofurantoïne, het middel van tweede keuze op basis van de antibioticagevoeligheidsresultaten, heeft een slechte penetratie in het prostaatweefsel, terwijl de prostaat vaak betrokken is bij een UWI bij mannen. Het zou daarom raadzaam zijn om klinische studies op te starten naar de effectiviteit van nitrofurantoïne als therapie voor een UWI bij de man.

Naast up-to-date behandelrichtlijnen, is ook een accurate diagnose van een UWI belangrijk voor het beheersen van de antibioticaresistentie. Om deze reden werd een diagnostisch algoritme ontwikkeld, gebaseerd op de voorspellende waarde van diagnostische informatie (klinische informatie en dipstick resulaten) voor een UWI bij een man (**hoofdstuk 6**). Een diagnostisch algoritme met daarin leeftijd boven de 60 jaar en een positieve dipstick test voor nitriet en leucocyten, was het meest accuraat in het voorspellen van een UWI bij een man. Dit algoritme had een sensitiviteit van 75% en een specificiteit van 70%, waarbij het gewenste voorschrijfbeleid op basis van de kweekresultaten als gouden standaard werd gebruikt. Het bleek echter, dat dit diagnostische algoritme niet superieur was ten op zichte van de dagelijkse praktijk, zoals weergegeven door de participerende huisartsen, in termen van sensitiviteit (75% versus 79%, *P*=0,30) en specificiteit (70% versus 63%, *P*=0,17).

Op basis van de diagnostische informatie was het wel mogelijk om een UWI te diagnosticeren, door de hoge mate van waarschijnlijkheid (≥90%) dat een UWI aanwezig was bij een positief resultaat, van voornamelijk de nitriet test. Het uitsluiten van een UWI bleek lastiger te zijn, door de relatief hoge waarschijnlijkheid (29%) van een UWI, zelfs wanneer alle voorspellende factoren voor een UWI afwezig waren. Hierdoor is meer onderscheidende diagnostische informatie voor een UWI bij de man nodig, met name voor het uitsluiten van deze aandoening.

Een significante antibioticadruk wordt ook uitgeoefend op de uropathogenen van vrouwen met recidiverende UWIs (rUWIs), omdat de huidige behandelrichtlijnen voor vrouwen die ten minste 3 UWIs binnen 12 maanden hebben doorgemaakt, langdurige antibiotische profylaxe in lage dosis voorschrijven. Om het aantal vrouwen bij wie deze profylactische behandeling geïndiceerd is, te verminderen, werd de voorspellende waarde van een asymptomatische bacteriurie (ASB) onderzocht op het optreden van een symptomatische UWI voor deze patiëntcategorie (**hoofdstuk 7**). Hier laten we zien dat de aanwezigheid van ASB geen voorspeller is voor het ontwikkelen van een UWI bij

vrouwen met rUWIs (hazard ratio=1,07, 95% betrouwbaarheidsinterval: 0,80–1,42). Hierdoor is de aanwezigheid van ASB niet geschikt voor het onderscheiden van vrouwen met rUWI die wel en die niet profiteren van de profylactische behandeling. Wel bleek dat de voorspellende waarde van het antibioticagevoeligheidspatroon van een *E. coli* ASB stam, één maand voorafgaand aan een *E. coli* UWI, ≥76% was voor alle geteste antibiotica, behalve in het geval van nitrofurantoïne- en co-amoxiclav-resistentie. Dit betekent dat het *E. coli* ASB gevoeligheidspatroon, één maand voor een *E. coli* UWI, meegenomen kan worden in de keuze voor empirische therapie bij vrouwen met rUWI, wat kan leiden tot een patiënt-specifiekere behandeling.

In **hoofdstuk 8** tonen we aan dat er verschillende determinanten zijn voor de aanwezigheid van antibioticaresistentie bij urine en faecale *E. coli* stammen, afkomstig van vrouwen met rUWI. De leeftijd van de patiënt was het best voorspellend voor faecale *E. coli* resistentie, terwijl het recente gebruik van trimethoprim of co-trimoxazol en het aantal UWIs in het voorafgaande jaar de belangrijkste determinanten waren voor urine *E. coli* resistentie. De gevonden determinanten konden alle verklaard worden door antibioticagebruik. De verschillen tussen urine en faecale *E. coli* resistentie kunnen het gevolg zijn van de specifieke pathogenese van rUWIs, met toenemend bewijs van de aanwezigheid van biofilms in de blaas.

LIST OF ABBREVIATIONS

APRES	The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance
ASB	Asymptomatic bacteriuria
ATC	Anatomical Therapeutic Chemical
ATCC	American type culture collection
BSI	Blood stream infections
BURP	Based upon repeat pattern
CA	Community-associated
CC	Clonal complex
cfu	Colony-forming units
CI	Confidence interval
CLSI	Clinical laboratory and standards institute
CR	Clinical recurrence
DID	Defined daily doses per 1000 inhabitants
EARSS	European Antimicrobial Resistance Surveillance System
EGPRN	European General Practice Research Network
ESAC	European Surveillance on Antimicrobial Consumption
ESBL	Extended-spectrum beta-lactamase
ESCAIDE	European Scientific Conference on Applied Infectious Disease Epidemiology
EUCAST	European committee on antimicrobial susceptibility testing
ExPEC	Extra-intestinal pathogenic *E. coli*
GDP	Gross domestic product
GI	Gastro-intestinal
GP	General practitioner
HA	Hospital-associated
HMP	Human Microbiome Project
HR	Hazard ratio
ICD-10	International Classification of Diseases, 10th revision
LE	Leukocyte esterase
LR+	Likelihood ratios for a positive test result
LR-	Likelihood ratios for a negative test result
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
MRSA	Meticillin-resistant *S. aureus*
NAPRUTI	Non-antibiotic prophylaxis for recurrent urinary tract infections
NHG	Dutch College of General Practitioners
NIVEL	The Netherlands Institute for Health Services Research
NPV	Negative predictive value

NVMM	Dutch Society of Medical Microbiology
OR	Odds ratio
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel-electrophoresis
PPV	Positive predictive value
PVL	Panton-Valentine leukocidin
ROC	Receiver operating characteristic
rUTI	Recurrent urinary tract infection
SCCmec	Staphylococcal cassette chromosome mec
SD	Standard deviation
spa	Staphylococcal protein A
SSTIs	Skin and soft-tissue infections
SWAB	Dutch Foundation of the Working Party on Antibiotic Policy
TMP/SMX	Trimethoprim/sulfamethoxazole
UK	United Kingdom
UTIs	Urinary tract infections
w/v	Mass/volume

DANKWOORD

Zonder goede medespelers is het afronden van een promotietraject een schier onmogelijke prestatie. Ik heb me gelukkig mogen prijzen dat ik een ideale mix van spelers om me heen heb gehad, die me hebben gesteund, meegeholpen en mij in kansrijke positie hebben gezet. Mijn proefschrift zou niet compleet zijn voordat ik iedere speler van het team in het zonnetje heb gezet.

Hierbij wil ik me ten eerste richten tot mijn copromotor, Ellen Stobberingh, die verantwoordelijk is geweest voor mijn directe begeleiding tijdens dit promotietraject. Lieve Ellen, jouw passie voor het vak Medische Microbiologie, en in het bijzonder de antibioticaresistentie, werkt aanstekelijk. Het feit dat jouw deur altijd openstond, zowel voor werkgerelateerde vragen als kwesties buiten de werkvloer, heeft ervoor gezorgd dat ik met groot plezier heb kunnen werken in mijn periode als promovendus. Terwijl je zelf liever op de achtergrond blijft, verzet je bergen werk voor de mensen om je heen. Op jou is de leus 'Bescheidenheid siert de mens' perfect van toepassing. Ook je grote persoonlijke betrokkenheid bij mij en mijn gezin heb ik enorm kunnen waarderen.

Mijn promotor Cathrien Bruggeman, als hoofd van de afdeling Medische Microbiologie: Beste Cathrien, ik wil u bedanken voor de interesse die u heeft getoond in mijn onderzoek en het bekijken van mogelijkheden voor mijn toekomst. Ook heb ik genoten van de gesprekken over onze (verre) reizen, hetgeen voor beiden van ons een grote hobby is. Ik hoop dat u na uw emiritaat nog genoeg mooie reizen kunt ondernemen en ook vollop kunt genieten van uw andere grote hobby, uw (klein) kinderen.

Ook wil ik mijn promotor en copromotor van het Nederlands instituut voor onderzoek van de gezondheidszorg (NIVEL), François Schellevis en John Paget, van harte bedanken voor mijn begeleiding. Mede door jullie is het APRES project succesvol verlopen, wat onlangs heeft geresulteerd in een mooie publicatie.

Resi, zonder jou was het logistieke traject van de APRES studie een stuk lastiger geweest. Jouw brede netwerk binnen het MUMC, in combinatie met jouw enthousiaste, vrolijke karakter, zorgt ervoor dat je veel voor elkaar kunt krijgen. Tevens hebben we als gezamenlijke hobby hardlopen, waarbij we vaak elkaars prestatie na het weekend hebben doorgenomen. Mijn vrouw omschreef jou, na jullie eerste kennismaking, als de ideale collega - en daar kan ik het alleen maar mee eens zijn.

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zijn jullie erin geslaagd om alle watten binnen het APRES project te analyseren en de antibioticagevoeligheidsbepalingen te verrichten, een prestatie van formaat! Daarnaast zijn jullie ook onmisbaar geweest voor de analyses binnen het SERIN en NAPRUTI project, waarvan de resultaten ook in dit proefschrift zijn gepresenteerd.

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John Penders, met jou heb ik een epidemiologie- en statistiekmaatje gevonden. Voor velen is dit niet het favoriete onderdeel van een onderzoekstraject, maar wij halen er veel voldoening uit. Jouw expertise heeft mij enorm geholpen bij het analyseren van de NAPRUTI data en natuurlijk ook bij het afronden van mijn Master Epidemiolgie.

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Via het NAPRUTI project, heb ik kennis gemaakt met Mariëlle Beerepoot, Suzanne Geerlings en Jan Prins. Het was enorm boeiend om met jullie te werken. Altijd hebben we goede, constructieve overleggen gevoerd, waaruit meerdere nieuwe onderzoeksvragen gedestilleerd konden worden. Onze samenwerking heeft tot nu toe geleid tot twee mooie publicaties en ik hoop het contact met jullie nog langdurig voort te zetten.

Als laatste wil ik hierbij Martien van Dongen noemen. Martien, op epidemiologisch gebied heb je me veel geleerd, zowel tijdens de opleiding tot epidemioloog als tijdens het werken aan ons artikel aangaande urineweginfecties bij mannen. Deze kennis zal me zeker van pas komen bij toekomstig wetenschappelijk onderzoek.

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Casper

CURRICULUM VITAE

Casper Dirk Johannes den Heijer werd geboren op 30 december 1981 te 's-Hertogenbosch. Op 5-jarige leeftijd verhuisde het gezin Den Heijer naar Eindhoven, alwaar hij in 2000 zijn VWO diploma behaalde aan het Van Maerlant Lyceum. Aansluitend begon hij de opleiding Geneeskunde aan de Universiteit van Maastricht. Zijn eerste wetenschappelijke ervaring deed hij op tijdens de wetenschapsstage binnen deze opleiding. Dit betrof het effect van roken op het ontstaan van de ziekte COPD (*chronic obstructive pulmonary disease*), onder begeleiding van professor Onno van Schayck en Annemiek Nijholt.

Na het behalen van zijn arts-examen op 25 september 2006, werkte hij 1 jaar als poortarts in het Sint Anna ziekenhuis te Geldrop. Hierna startte hij met de opleiding Huisartsgeneeskunde. Tijdens deze opleiding ontdekte hij echter dat zijn passie meer bij de theoretische kant van de geneeskunde lag, en hij besloot de opleiding voortijdig te beëindigen. Na contact met Ellen Stobberingh, begon hij op 1 oktober 2009 als promovendus aan de afdeling Medische Microbiologie van het Maastricht Universitair Medisch Centrum. Hij werd aangesteld op het project '*The appropriateness of prescribing antibiotics in primary care in Europe with respect to antibiotic resistance* (APRES)'. Tijdens zijn promotietraject doorliep hij de Master Epidemiologie aan de Universiteit Maastricht, waarin hij op 29 februari 2012 afstudeerde met het predikaat Cum Laude. Op dit moment is hij werkzaam op de afdeling Medische Microbiolgie waar hij zich bezig houdt met statistische analyses met behulp van het programma SAS, het uitvoeren van analyses in de bioinformatica en het schrijven van artikelen aan de hand van data voortgekomen uit het APRES project. Hij is woonachtig in Sittard waar hij samenwoont met zijn vrouw Marjolein en zoon Max.

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